American

The Journal of

March-April 1954

VOLUME 2 — NUMBER 2

CLINICAL NUTRITION

AN INTERNATIONAL JOURNAL REPORTING THE PRACTICAL APPLICATION OF OUR NEWER KNOWLEDGE OF NUTRITION

CONTENTS

PUBLISHED BIMONTHLY BY
THE NUTRITIONAL PRESS
ALLENTOWN, PENNSYLVANIA

The Journal of Clinical Nutrition

AN INTERNATIONAL JOURNAL REPORTING THE PRACTICAL APPLICATION OF OUR NEWER KNOWLEDGE OF NUTRITION

The Journal of Clinical Nutrition

AN INTERNATIONAL JOURNAL REPORTING THE PRACTICAL APPLICATION OF OUR NEWER KNOWLEDGE OF NUTRITION

CONTENTS

PUBLISHED BIMONTHLY BY
THE NUTRITIONAL PRESS
ALLENTOWN, PENNSYLVANIA

THE JOURNAL OF CLINICAL NUTRITION

MARCH–APRIL, 1954 VOLUME 2, NUMBER 2

NUTRITIONAL STUDIES OF VEGETARIANS*

1. NUTRITIONAL, PHYSICAL, AND LABORATORY STUDIES

By MERVYN G. HARDINGE, M.D.,† AND FREDRICK J. STARE, M.D.

Additional knowledge as to the nutritional requirements of human beings may be gained by a study of those who have unique dietary habits. The vegetarian groups in the United States afford an opportunity to observe the effect of such "natural experiments." Experimental studies in this field have generally been for short periods of time, and of the few surveys made many were conducted in areas of the world where poor economic conditions greatly curtail the amount and type of foods eaten. It was, therefore, believed that a comparative study of vegetarians, made in times and areas of relative plenty, among people who have voluntarily selected their dietary regimes, and who have pursued these practices for years, lifetimes, and even generations, would contribute valuable information to the present knowledge of nutrition.

Mention should be made of the few surveys and group studies conducted among vegetarians. Yukawa[1] studied a group of vegetarian monks in Japan and found them to be in good health. He estimated that their diets, if evaluated for a 150-pound male, supplied 3575 calories and 113 grams of protein. The older men ate somewhat less. The diet was chiefly rice and barley, with soy products, vegetables, and rape seed oil. Orr[2] compared the diet of two African tribes and found that those who consumed meat were taller, healthier, and had less anemia than those who did not. The diet of the former group contained milk, meat, and blood, in addition to maize, millet, plantains, and yams, which comprised the diet of the latter group. Thus, the diet of the vegetarian tribe was markedly restricted in variety.

Taylor[3] reported a study of two groups of soldiers stationed in the Near East; one group used meat in their diet while the other did not. The incidence of macrocytic anemia was significantly higher among the vegetarians, although iron deficiency anemia existed among both groups. Limitation in the availability of fruits, vegetables, and milk which was to replace the meat in the vegetarian diet handicapped the vegetarian especially.

In this country Jaffa[4] studied a family of fruitarians in California, and found that the family was in apparently good health, although underweight. The children were somewhat small in stature as compared to American children of their age and sex. More recently, Foote and Eppright[5] investigated the

From the Department of Nutrition, Harvard School of Public Health, Boston.

* The data presented in this paper represent partial fulfillment (by M.G.H.) of the requirements for the degree of Doctor of Public Health at the Harvard School of Public Health.

† School of Medicine, College of Medical Evangelists, Loma Linda, California.

Supported in part by a grant-in-aid from the Nutrition Foundation, New York.

dietary of boys and girls in a lacto-ovo-vegetarian regime. It was found that the caloric intakes of the children were slightly under recommended allowances, but their health was good, and their weights and heights compared favorably with the "standards." Mirone[6] studied a group of lacto-ovo-vegetarians, who used no meat and only small quantities of dairy products. Hematologic and blood protein determinations were made. The findings were all within the normal range. Oldham and Sheft[7] studied the effect of caloric intake on nitrogen utilization of lacto-ovo-vegetarians and non-vegetarians during pregnancy. No significant differences were observed.

Selection of Subjects

It has been estimated by Gallup[8] (1943) that there are two and one-half to three million vegetarians in the United States. These are divided among three widely separate and distinct vegetarian groups. One is found within the ranks of the Catholic Church, the Trappist monks. Their dietary usually includes the use of milk but no meat. Another is sponsored by the Seventh-day Adventist Church, whose health and educational program includes a lacto-ovo-vegetarian diet. The third vegetarian group is heterogenous in nature, being drawn together by various motives such as nonviolence and animal friendliness. A few persons in each group, mainly among the last, exclude all foods of animal origin and are here referred to as "pure" vegetarians.

The subjects selected for this study were adolescents, pregnant women, and men and women 45–70 years of age. The latter were included in an effort to discover the effects of such dietary regimes maintained over long periods of time. The pregnant women and adolescents were "stress" groups, and were most likely to reveal inherent weaknesses in their dietaries. Three classes of subjects were sought for each group, namely, lacto-ovo-vegetarians, "pure" vegetarians, and non-vegetarians. Lacto-ovo-vegetarians are here defined as individuals who do not use as food the flesh of animals (meat, poultry, and fish), but do eat such foods as milk, eggs, and products made from or containing milk and eggs. "Pure" vegetarians are those who exclude from their diets all foods of animal origin.

The following means were employed to locate subjects. The adult vegetarian subjects were located through questionnaires circulated in commercial and sectarian magazines and among church and social groups where they might be found, and the adult non-vegetarians were obtained from similar groups. Adolescents were found through questionnaires distributed to students in both private and public high schools with the co-operation of the administrators. The majority of expectant mothers were contacted through their physicians.

The vegetarian groups, as far as possible, were selected first, and then from the much larger population of non-vegetarians the respective control groups were formed. Age, because of its tangible nature, was used as the criterion for the latter selection, the ages of subjects in respective groups being approximately matched. In selecting the expectant mothers, all lacto-ovo-vegetarian pregnant women who qualified were chosen without regard to age or number of pregnancies. Likewise these factors were not considered when the non-vegetarian control groups were selected.

All subjects were of the white race, of average or above average social and economic levels, and considered themselves to be in good health. All had voluntarily maintained their respective dietaries from a minimum of five years preceding the study to throughout life, and with few exceptions were residents of Central and Southern California. As indicated in Table I, a total of 200 subjects who fulfilled the general group specifications were studied, of which 86 were lacto-ovo-vegetarians, 26 "pure" vegetarians, and 88 non-vegetarians.

Methods

The dietary history method of Burke[9] is well suited to determine an individual's long-term levels and patterns of food intake and was used in the present investigation. The fact that the vegetarians, especially the "pure" vegetarians, tend to be "food conscious" was

TABLE I

Age Distribution and Duration on Dietary Regime of Subjects by Groups

Classes and groups	Number of subjects	Age* Mean	Age* Range	Duration on diet, mean
ADULT				
Males				
L-o-vegetarian†	15	55.0	42–66	Life
Pure vegetarian	14	51.0	23–80	16 years‡
Non-vegetarian	15	56.5	45–69	Life
Females				
L-o-vegetarian	15	57.5	45–71	Life
Pure vegetarian	11	48.5	25–68	9 years‡
Non-vegetarian	15	56.5	45–73	Life
ADOLESCENT				
Males				
L-o-vegetarian	15	15.5	14–17	Life
Pure vegetarian	1	17.0		Life
Non-vegetarian	15	15.5	14–17	Life
Females				
L-o-vegetarian	15	14.0	13–16	Life
Pure vegetarian				
Non-vegetarian	15	14.0	13–16	Life
PREGNANT WOMEN				
L-o-vegetarian, lifetime	12	25.5	20–32	Life
L-o-vegetarian, long-term	14	26.0	22–34	10 years‡
Total	26	26.0	20–34	
Non-vegetarian	28	25.5	17–38	Life
GRAND TOTAL	200			

* Age, in years, of subject at last birthday.

† L-o-vegetarian is one on a lacto-ovo-vegetarian diet. See text.

‡ Subjects had maintained their respective diets for the last five or more years.

a definite advantage in the present study. To facilitate the computation of the dietaries special tables were prepared, the data for which were obtained from several standard sources.[10–12]

In the medical history, information was secured as to the subject's general health, both past and present. Attention was focused on the presence or absence, not alone in the individual but also in his immediate blood relatives, of disease, especially disorders which might have some nutritional significance.

A detailed record of the present pregnancy of the expectant mothers was made. Changes of weight, expected date of delivery, and complicating illnesses were noted. History of premature births, miscarriages, and fertility were recorded. Following delivery, the details of duration of labor, infant and maternal weights, complications of labor if any, and nursing record were obtained.

One of the authors (M.G.H.) made a thorough physical examination of each subject with careful measurement of height, weight, blood pressure, and the presence of any pathological conditions, especially any which might be indicative of nutritional deficiency. The following laboratory procedures were carried out on each subject, using venous blood: the packed-cell volume by the method of Wintrobe,[13] erythrocyte, leukocyte, and differential white blood cell counts by standard procedures; and the measurements of total serum protein, albumin, and globulin by specific gravity methods described by Mortensen.[14,15]

Results

Dietary Findings

In general the lacto-ovo-vegetarian pattern of eating closely parallels the eating habits of the average American except for the exclusion of meat. The parents of most of the subjects had become vegetarians some time prior to the birth of their children. As might be expected, the eating habits of these subjects were modified in certain respects, mainly substituting for the flesh of animals a more generous intake of milk, cheese, eggs, legumes, and to a lesser extent nuts and commercially prepared nut foods ("meat substitutes").

Variations in the dietaries of the "pure" vegetarians were marked and food consciousness was very evident. The usual dietary included cereals—cooked and in the form of bread—legumes, nuts, large quantities of fruits and vegetables, especially large vegetable salads, vegetable oils, and olives. Minimal quantities of refined and commercially prepared foods were used. Few desserts were eaten, although most of the subjects consumed liberal quantities of honey. As a group, they ate moderate quantities of dark and black strap molasses, while sesame and sunflower seeds were frequently incorporated in the cooking or ground to make butters. Tea, coffee, chocolate, soft drinks, and alcoholic beverages

TABLE II

Average Daily Dietary Intake of Vegetarian and Non-vegetarian Groups

Groups	No.	Calories	Protein			Minerals					Vitamins				
						Calcium									
			Animal	Plant	Total	Animal	Plant	Total*	Phos.	Iron	A	C	B_1	B_2	Niacin
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*mg.*	*I.U.*†	*mg.*	*mg.*	*mg.*	*mg.*
ADULT															
Males															
L-o-vegetarian	15	3020	41	57	98	1.0	0.7	1.7	2.2	22	15,400	250	2.3	2.8	19
Pure vegetarian	14	3260	—	83	83	—	1.1	1.1	1.9	30	25,570	355	2.7	1.8	26
Non-vegetarian	15	3720	86	39	125	1.0	0.4	1.4	2.2	22	14,420	185	2.0	2.8	23
Females															
L-o-vegetarian	15	2450	43	39	82	1.1	0.5	1.6	1.8	16	13,470	220	1.7	2.6	13
Pure vegetarian	11	2400	—	61	61	—	0.9	0.9	1.4	25	19,510	280	2.1	1.5	16
Non-vegetarian	15	2690	64	30	94	0.6	0.4	1.0	1.6	17	13,730	185	1.5	2.1	18
ADOLESCENT															
Males															
L-o-vegetarian	15	4450	69	72	141	1.9	0.7	2.6	3.1	25	17,920	210	3.0	4.2	18
Pure vegetarian	1	4360	—	56	56	—	1.1	1.1	1.6	29	16,790	235	2.0	1.5	20
Non-vegetarian	15	5350	116	63	179	1.8	0.6	2.4	3.3	28	17,230	185	2.8	4.2	33
Females															
L-o-vegetarian	15	3030	51	49	100	1.2	0.5	1.7	2.0	18	16,380	185	2.2	3.0	16
Pure vegetarian	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Non-vegetarian	15	4100	106	45	151	1.7	0.5	2.2	2.8	23	16,820	210	2.4	3.9	26
PREGNANT WOMEN															
L-o-vegetarian	26	2650	56	41	97	1.3	0.5	1.8	2.2	17	15,110	205	1.9	3.0	14
Pure vegetarian	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Non-vegetarian	28	3010	81	30	111	1.2	0.3	1.5	2.0	16	12,430	155	1.7	2.8	18
CHILD (Boy, age 10)															
Pure vegetarian	1	3550	—	48	48	—	0.8	0.8	1.2	20	10,750	165	1.7	1.2	17

* Calcium from plants high in oxalic acid not included.

† Includes carotene

were rarely used, although it was common to use herb teas. Perhaps an outstanding characteristic of the group was the consumption of large quantities of fruit and vegetable juices.

The non-vegetarians were included for the purpose of providing a control or standard group. Since the eating and living habits of this group are well known, no detailed description will be given.

The average dietary of the lacto-ovo-vegetarian and non-vegetarian groups (Table II) provided the various nutrients in amounts recommended by the National Research Council.[16] However, in the case of a few individuals the intakes of certain nutrients were below the suggested allowances. Almost half of the lacto-ovo-vegetarian and non-vegetarian adult men and women, and half of the lacto-ovo-vegetarian pregnant women had caloric intakes below those recommended. Individual non-vegetarians, especially among the expectant mothers, were below recommended allowances in calcium and riboflavin, while certain lacto-ovo-vegetarians had niacin intakes below those suggested.

The average dietary of the "pure" vegetarian adult groups supplied most nutrients in amounts recommended, a few subjects having intakes of protein, calcium, and riboflavin below the allowances suggested by the National Research Council. Due to the great scarcity of "pure" vegetarians, we were able to study only small samples of the adult group. It was not possible to find groups of "pure" vegetarian adolescents and pregnant women, because few parents themselves had maintained such a dietary throughout the lifetime of their children, and because in times of physiologic stress (as pregnancy) "pure" vegetarians often revert to a diet which includes milk and eggs.

Only one adolescent, a 17-year-old boy, was located who had been a "pure" vegetarian throughout life. Both he and his 10-year-old brother, similarly raised, appeared normal and healthy. Their dietary intakes are included in Table II.

Physical Findings

Comparison of the heights and weights of all groups of lifetime lacto-ovo-vegetarians and non-vegetarians showed no significant differences (Tables III and IV). The "pure" vegetarian groups, both men and women, showed a mean weight 20 pounds lower than their respective lacto-ovo-vegetarian or non-vegetarian groups, despite the fact that the caloric intakes of the three groups appeared to be approximately the same.

The pregnant women, lacto-ovo-vegetarian and non-vegetarian, whether primiparae or multiparae, had gained an average of 22 pounds at term, returning to their mean preconception weights within the third week post partum. All of the deliveries were without serious complications. The average birth weights and birth lengths of the two groups of infants were not statistically different, except that the mean birth weight of infants born to

TABLE III

Comparison of Heights of Lacto-ovo-vegetarian and Non-vegetarian Groups

Groups	L-o-vegetarian			Non-vegetarian			Diff. of Means	S.E. of Diff.†
	No.	Height, Mean	S.D.*	No.	Height, Mean	S.D.*		
		In.			*In.*			
ADULT								
Males	15	68.0	2.81	15	69.5	3.74	1.5	1.21
Females	15	62.5	2.56	15	64.0	1.79	1.5	0.80
ADOLESCENT								
Males	15	68.0	3.19	15	68.5	3.31	0.5	1.19
Females	15	64.0	3.91	15	63.0	2.91	−1.0	1.26
PREGNANT WOMEN	12	64.5	2.65	28	64.5	3.38	0.0	0.93

* Standard Deviation.

† Standard Error of Difference = $\sqrt{(\sigma_1^2/n_1 - 1) + (\sigma_2^2/n_2 - 1)}$.

TABLE IV

Comparison of Weights of Vegetarian and Non-vegetarian Groups

Groups	Lo-vegetarian Group I			Pure vegetarian Group II			Non-vegetarian Group III			Comparison of Groups							
										I & III		II & III		I & II			
	No.	Weight, Mean	S.D.	No.	Weight, Mean	S.D.	No.	Weight, Mean	S.D.	Diff. of Means	S.E. of Diff.	Diff. of Means	S.E. of Diff.	Diff. of Means	S.E. of Diff.
		Lb.			*Lb.*			*Lb.*							
ADULT															
Males	15	162	19.8	14	146	24.3	15	170	28.4	8	8.8	24	9.8	16	8.2
Females	15	138	26.7	11	117	19.9	15	142	20.6	4	8.1	25	7.8	21	9.0
ADOLESCENT															
Males	15	141	14.6	—	—	—	15	142	27.5	* 1	8.0	—	—	—	—
Females	15	117	21.2	—	—	—	15	112	10.4	5	6.1	—	—	—	—
PREGNANT WOMEN	26	130	16.3	—	—	—	28	129	17.6	1	4.5	—	—	—	—

the non-vegetarian primiparous mothers was slightly more than that of the other groups.

In general the blood pressures of the lacto-ovo-vegetarian, "pure" vegetarian, and non-vegetarian groups showed no significant difference. One adult male and six adult female lacto-ovo-vegetarians and five adult female non-vegetarians had systolic blood pressures above 150 millimeters mercury. As a result, the average systolic pressures of the lacto-ovo-vegetarian and non-vegetarian adult women were in the upper limit of the normal range.

Certain minor pathological findings were observed scattered among all groups, such as acne vulgaris, dental caries, and varicosities. However, little of a comparative nature is present. The subjects all enjoyed good health.

Laboratory Findings

No subject was included in the study for whom there was no accompanying laboratory work. Many "pure" vegetarians for various reasons were unwilling to permit laboratory measurements, and as a result the sample studied is smaller than that of the other groups.

In Table V, the hemoglobin, hematocrit, and erythrocyte counts for each of the several groups have been summarized, together with certain corpuscular values. When the vegetarian groups are compared with their control groups only slight differences are observed, the values being within the accepted normal ranges. Of the seventeen subjects with hemoglobin values below 12 grams per 100 ml. (Table VI) there were 8 lacto-ovo-vegetarians, 8 non-vegetarians, and one "pure" vegetarian. Leukocyte and differential counts of all groups were well within normal limits.

The total protein, albumin, and globulin measurements for all groups studied are shown in Table VII. The differences between the respective groups are not statistically significant. The pregnant women show a moderate reduction in their total protein and albumin concentrations. This is in accord with the findings of Rinehart[17] and Wiehl.[18]

Discussion

Most vegetarians and non-vegetarians were "protein conscious" and made every effort to

TABLE V

Comparison of Hematological Findings of Vegetarian and Non-vegetarian Groups

Groups	No.	Hemoglobin		Hematocrit Volume		Erythrocyte Count		M.C.V.*	M.C.H.C.†	M.C.H.‡
		Mean	S.D.	Mean	S.D.	Mean	S.D.			
		Gm./100 ml.		%	%	*Millions/mm.*^{3}			%	
ADULT										
Males										
L-o-vegetarian	15	15.8	1.57	44.1	2.27	5.41	0.58	82	36	29
Non-vegetarian	15	15.6	0.96	43.6	3.04	5.37	0.62	81	36	29
Pure vegetarian	14	15.1	2.25	42.8	3.25	4.84	0.59	88	35	31
Females										
L-o-vegetarian	15	13.6	1.75	39.1	1.82	4.75	0.34	82	35	29
Non-vegetarian	15	13.2	1.15	39.5	2.31	4.72	0.93	84	34	28
Pure vegetarian	11	13.8	2.02	39.1	3.38	4.48	0.58	87	37	31
ADOLESCENT										
Males										
L-o-vegetarian	15	14.9	1.34	42.5	3.13	5.34	0.34	80	35	28
Non-vegetarian	15	15.0	0.78	42.0	1.64	5.26	0.37	80	36	29
Pure vegetarian	1	12.6		35.0		3.64		96	36	35
Females										
L-o-vegetarian	15	13.2	2.34	38.2	3.54	4.74	0.59	81	35	28
Non-vegetarian	15	13.1	0.93	39.0	2.72	4.78	0.32	82	34	27
PREGNANT WOMEN										
L-o-vegetarian	26	11.9	1.23	34.5	2.57	4.28	0.49	81	35	29
Non-vegetarian	28	12.2	0.95	35.6	2.77	4.19	0.45	85	34	29

* M.C.V. = Mean corpuscular volume.

† M.C.H.C. = Mean corpuscular hemoglobin concentration.

‡ M.C.H. = Mean corpuscular hemoglobin.

TABLE VI

Vegetarian and Non-vegetarian Subjects with Hemoglobin Levels Below 12 Gm. Per 100 ml.

Subjects	Hb.*	Het.†	R.B.C.‡	M.C.V.**	M.C.H.C.**	M.C.H.**
ADULT						
Males						
Pure vegetarian						
Subject #1	11.5	43.0	4.10	101	27	28
Females						
L-o-vegetarian						
Subject #88	11.6	34.0	4.81	71	34	24
Non-vegetarian						
Subject #196	11.2	42.0	4.55	92	27	25
Subject #197	11.4	43.0	4.96	87	27	23
Subject #204	11.4	35.0	4.93	71	33	23
ADOLESCENT						
Females						
L-o-vegetarian						
Subject #26	11.6	33.0	4.08	81	33	28
Subject #73	10.5	31.5	4.38	72	33	24
Non-vegetarian						
Subject #99	11.9	36.0	4.58	79	33	26
Subject #116	11.7	38.0	4.45	85	31	26
Subject #119	11.5	35.0	4.49	78	33	26
PREGNANT WOMEN						
L-o-vegetarian						
Subject #35	10.4	32.5	4.05	80	32	26
Subject #37	8.8	27.5	3.87	71	32	28
Subject #43	10.6	33.5	4.33	74	32	25
Subject #46	10.9	34.0	4.63	73	32	24
Subject #166	10.4	31.0	3.36	92	34	31
Non-vegetarian						
Subject #107	10.8	30.5	3.33	92	35	32
Subject #137	10.4	31.5	3.74	84	33	28

* Hb. = Hemoglobin (Gm./100 ml.).
† Het. = Hematocrit (Volume %).
‡ R.B.C. = Red Blood Count (millions/mm.^{3}).
** See footnote—Table V.

ensure a liberal intake of this nutrient. The non-vegetarian adolescents consumed significantly more protein than did the lacto-ovo-vegetarian adolescents; however, this larger protein intake was not reflected in greater growth as measured by height. No evidence was obtained to indicate that a lacto-ovo-vegetarian diet failed to provide the dietary standards recommended for adequate nutrition for an expectant mother.

The adult non-vegetarian men and women were slightly taller than their respective lacto-ovo-vegetarian groups, but this difference was not significant. It is possible that it might be related to the diet of the older generation. However, no such differences are noted in stature of the respective groups of the younger generation.

It is of interest that the "pure" vegetarian men and women had significantly lower weights, averaging 20 pounds less than adults in the other two groups, despite the fact that their caloric intakes and physical activities were approximately the same. Ling and Chow[19] observed that rats on a diet deficient in vitamin B_{12} had decreased quantities of carcass fat which could be restored by administration of this vitamin. This weight increase was not associated with any detectable increase in nitrogen retention, but rather suggests greater efficiency in caloric utilization. This provides a possible explanation for our finding.

TABLE VII

Comparison of Total Protein, Albumin, and Globulin Levels of Vegetarian and Non-vegetarian Groups

Groups	No.	Total Protein		Albumin		Globulin	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
		Gm./100 ml.		*Gm./100 ml.*		*Gm./100 ml.*	
ADULT							
Males							
L-o-vegetarian	15	7.4	0.51	5.1	0.46	2.3	0.47
Pure vegetarian	14	7.4	0.46	5.0	0.57	2.4	0.47
Non-vegetarian	15	7.2	0.30	5.0	0.58	2.2	0.52
Females							
L-o-vegetarian	15	7.5	0.35	4.7	0.52	2.8	0.32
Pure vegetarian	11	7.2	0.52	4.9	0.54	2.3	0.35
Non-vegetarian	15	7.3	0.47	5.1	0.41	2.2	0.52
ADOLESCENT							
Males							
L-o-vegetarian	15	7.4	0.25	5.2	0.46	2.2	0.36
Non-vegetarian	15	7.6	0.40	5.6	0.49	2.0	0.33
Females							
L-o-vegetarian	15	7.4	0.35	5.1	0.44	2.3	0.37
Non-vegetarian	15	7.5	0.52	5.6	0.43	1.9	0.46
PREGNANT WOMEN							
L-o-vegetarian	26	6.3	0.35	4.0	0.47	2.3	0.30
Non-vegetarian	28	6.5	0.34	4.4	0.45	2.1	0.46

There was no significant association between the blood pressure and a plant dietary. It is of interest that despite the lower protein intakes of the vegetarian groups there was no lowering of the serum protein values even among the "pure" vegetarian groups who had utilized a relatively lower all-plant diet an average of 9 to 16 years. Since the protein intake of all the vegetarian groups was almost equal to or exceeded the National Research Council's recommended allowances, it appears that above a certain level of dietary protein intake, whether of plant or animal origin, the level of serum protein concentration is not measurably affected.

The per cent of subjects having hemoglobin levels below 12 Gm. per cent was approximately the same in all three groups. There appears to be a tendency for the pure vegetarians to have mean corpuscular volumes somewhat higher than the lacto-ovo-vegetarians and non-vegetarians.

Summary

A comparative study of 112 vegetarian and 88 non-vegetarian adults, adolescents, and pregnant women is described. The results show that although the dietary intake of nutrients varied widely among individuals, the average intake of all groups, with the exception of the adolescent "pure" vegetarian, approximated or exceeded the amounts recommended by the National Research Council. Non-vegetarian adolescents consumed significantly more protein than did lacto-ovo-vegetarian and "pure" vegetarian adolescents. No evidence was obtained to indicate that a lacto-ovo-vegetarian diet failed to provide an adequate dietary for an expectant mother.

In general, measurements of height, weight, and blood pressures of these groups showed no significant differences. However, the "pure" vegetarians weighed appreciably less, an average of 20 pounds. Preconception and post partum weight gains and losses of the pregnant women were similar, as were the average birth weights of infants among the lacto-ovo-vegetarians and non-vegetarians.

The total protein, albumin, and globulin values, and the hematological findings for all the vegetarian and non-vegetarian groups were not statistically different.

Acknowledgment

The senior author wishes to express his appreciation to Mrs. Bertha S. Burke, Dr. Robert B. Reed, and Dr. George V. Mann for their help and advice during the course of this study.

REFERENCES

1. Yukawa, G.: The absolute vegetarian diet of Japanese Bonzes. *Arch. Verdauungskr.* 15: 471, 1909.
2. Orr, J. B., and Gilks, J. L.: Studies in nutrition: The physique and health of two African tribes. *Medical Research Council, Special Report Series* No. 135, Her Majesty's Stat. Off., London, 1931.
3. Taylor, O. F., Chhuttani, P. N., and Kumar, S.: The meat ration and blood levels. *Brit. M. J.* 4596: 219, 1949.
4. Jaffa, M. E.: Nutrition investigation among fruitarians and Chinese. *U. S. Dept. Agric. Bull.* No. 107, 1901.
5. Foote, R., and Eppright, E. S.: Dietary study of boys and girls on lacto-ovo-vegetarian diet. *J. Am. Dietet. A.* 16: 222, 1940.
6. Mirone, L.: Blood findings in men on a diet devoid of meat and low in animal protein. *Science* 111: 673, 1950.
7. Oldham, H., and Sheft, B. B.: Effect of caloric intake on nitrogen utilization during pregnancy. *J. Am. Dietet. A.* 27: 847, 1951.
8. Gallup, G.: The Gallup poll. *Washington Post,* October 2, 1943.
9. Burke, B. S.: Dietary history as a tool in research. *J. Am. Dietet. A.* 23: 1041, 1947.
10. Watt, B. K., and Merrill, A. L.: Composition of foods—raw, processed, prepared. *Agriculture Handbook* No. 5, U. S. Department of Agriculture, 1950.
11. Bowes, A. D., and Church, C. F.: *Food Values of Portions Commonly Used.* Published by Senior Author, Philadelphia, 1951.
12. Platt, B. S.: Tables of representative values of foods commonly used in tropical countries. *Medical Research Council, Special Report* No. 253, Her Majesty's Stat. Off., London, 1945.
13. Wintrobe, M. M.: The size and hemoglobin content of the erythrocyte. *J. Lab. & Clin. Med.* 17: 899, 1932.
14. Mortensen, R. A.: A rapid method for the determination of serum protein. *J. Lab. & Clin. Med.* 27: 693, 1942.
15. Mortensen, R. A., and Hardinge, M.: The determination of albumin and globulin in blood serum by specific gravity measurements. *J. Lab. & Clin. Med.* 28: 1649, 1943.
16. Food and Nutrition Board: Recommended dietary allowances (revised). *Reprint Series 129,* National Research Council, Washington, D. C., 1948.
17. Rinehart, R. E.: Serum protein in normal and toxemic pregnancy. *Am. J. Obst. & Gynec.* 50: 48, 1945.
18. Wiehl, D. G.: Changes in blood values during pregnancy and the relation of protein levels to toxemia symptoms. *Milbank Mem. Fund Quart.* 28: 238, 1950.
19. Ling, C. T., and Chow, B. F.: Effect of vitamin B_{12} on the body composition of rats. *J. Biol. Chem.* 198: 439, 1952.

Resumen

Estudios nutricionales de vegetarianos. 1. Estudios nutricionales, físicos y de laboratorio

Se describe un estudio comparativo de 112 adultos vegetarianos y 88 no vegetarianos, adolescentes y mujeres encintas. Los resultados demuestran que aunque la ingesta dietética de substancias nutritivas variaba grandemente entre los indivíduos, el término medio de ingesta en todos los grupos, con excepción de los adolescentes vegetarianos "puros," se aproximaba o excedía de las cantidades recomendadas por el Consejo Nacional de Investigación. Los adolescentes no vegetarianos consumían cantidades significantemente mayores de proteínas que los adolescentes lacto-ovo-vegetarianos y vegetarianos "puros." No se encontró evidencia de que la dieta lacto-ovo-vegetariana falle en producir una ingesta dietética adecuada para la mujer encinta.

En general, en los diversos grupos, no se encontraron diferencias mayores en las medidas de talla, peso y presión arterial. Sin embargo, los vegetarianos "puros" pesaban apreciablemente menos, un término medio de 20 libras. Los aumentos y pérdidas de peso preconcepcionales y post partum de las mujeres encintas, así como también el peso medio de los infantes al nacer fueron similares entre los grupos lacto-ovo-vegetarianos y no vegetarianos.

La proteína total, los valores de albúmina y globulina así como los estudios hematológicos para todos los de los grupos vegetariano y no vegetariano, no fueron estadísticamente diferentes.

NUTRITIONAL STUDIES OF VEGETARIANS*

2. DIETARY AND SERUM LEVELS OF CHOLESTEROL

MERVYN G. HARDINGE, M.D.† AND FREDRICK J. STARE, M.D.

IN THE United States the vegetarian groups afford a singular opportunity to compare dietary intakes and serum cholesterol concentrations with those of control groups. Eighty-six lacto-ovo-vegetarian, twenty-six "pure" vegetarian, and eighty-eight non-vegetarian adults, adolescents, and pregnant women were studied. Lacto-ovo-vegetarians include milk and eggs in their diet but do not eat flesh of animals (meat, poultry, fish). "Pure" vegetarians eat no foods of animal origin. Details regarding the selection and composition of these groups, together with a report on their dietary practices, physical condition, and laboratory findings are described in the preceding paper. This study discusses the cholesterol findings.

METHODS

Tables for the estimation of cholesterol in the diets were prepared from data reported by Okey[1] and reproduced in part by Turner,[2] while dietary fat was calculated from the food composition tables of the United States Department of Agriculture[3] and Bowes and Church.[4] Serum cholesterol was determined by the method of Bloor.[5]

From the Department of Nutrition, Harvard School of Public Health, Boston.

*The data presented in this paper represent partial fulfillment (by M.G.H.) of the requirements for the degree of Doctor of Public Health at the Harvard School of Public Health.

†School of Medicine, College of Medical Evangelists, Loma Linda, California.

Supported in part by a grant-in-aid from the Nutrition Foundation, New York.

RESULTS

The caloric consumption and levels of fat and cholesterol in the diets of the various groups have been summarized in Table I. The average calories derived from total dietary fat approximate the estimate of 38 per cent for the United States made by the Food and Agriculture Organization.[6] In Table II, the per cent of calories obtained from total fat, together with that fraction furnished by animal fat alone, are compared between corresponding groups. The non-vegetarians have significantly higher intakes in both instances.

The dietary cholesterol of the non-vegetarians is definitely greater than that of the lacto-ovo-vegetarians. The "pure" vegetarians, because of exclusion of all foods of animal origin, have essentially cholesterol-free diets. The serum cholesterol levels of these groups are compared in Table III. No significant differences exist between the lacto-ovo-vegetarian and non-vegetarian groups, with the exception of the adult lacto-ovo-vegetarian males, who have a significantly lower value. When the lower serum cholesterol concentrations of the "pure" vegetarian groups are compared with their respective lacto-ovo-vegetarian and non-vegetarian groups, a high degree of significance is observed. The cholesterol levels of the expectant mothers are not excessive, as there is customarily a rise in blood cholesterol concentration during pregnancy.

DISCUSSION

At the outset, it should be mentioned that the number of subjects in this study is small.

TABLE I

Caloric, Fat, and Cholesterol Intake of Vegetarians and Non-vegetarians

Group	No.	Total calories	Total fat	Calories from fat	Cholesterol	
					Total	Mg./100 calories
			Gm.	%	*mg.*	
ADULTS						
Males						
L-o-vegetarian*	15	3020	107.6	33.1	333	11.0
"Pure" vegetarian	14	3260	130.2	35.2	0	
Non-vegetarian	15	3720	175.5	43.0	914	24.6
Females						
L-o-vegetarian	15	2450	92.2	33.3	350	14.3
"Pure" vegetarian	11	2400	96.9	34.2	0	
Non-vegetarian	15	2690	124.2	41.7	612	22.8
ADOLESCENT						
Males						
L-o-vegetarian	15	4450	167.4	34.0	599	13.5
Non-vegetarian	15	5350	246.6	41.3	1046	19.5
Females						
L-o-vegetarian	15	3030	118.9	34.5	408	13.5
Non-vegetarian	15	4100	192.6	41.8	829	20.2
PREGNANT WOMEN						
L-o-vegetarian	26	2650	96.1	32.6	464	17.5
Non-vegetarian	28	3010	132.0	39.5	627	20.8

* Lacto-ovo-vegetarian. See text.

Because of that, the results reported can only be considered as possibly suggesting interesting trends.

The daily intake of exogenous cholesterol by the lacto-ovo-vegetarians and non-vegetarians is suprisingly different. This difference is explained by considering the sources of cholesterol in the diets. The use of eggs by both groups is approximately the same. Although the lacto-ovo-vegetarians do consume more milk than the non-vegetarians, yet to the cholesterol obtained by the non-vegetarians from dairy products must be added the significant amount contained in various meats, fish, and fowl. As recently pointed out by Phil,[7] a liter of milk contains approximately 135 milligrams of cholesterol, a quantity which may be smaller than that contributed by the average serving of meat eaten at dinner (100–150 mg.).

The cholesterol-free diet of the "pure" vegetarian is of special interest because of the use in the past decade of low cholesterol diets for individuals with atherosclerosis. The observations[8] that dietary fat, whether of animal or vegetable origin, may influence the blood cholesterol level and the investigations of several workers [9–11] using diets low in both cholesterol and fat have indicated that serum cholesterol levels may reflect the fat rather than the cholesterol intake. In this study, there exists a direct quantitative correlation between blood cholesterol concentrations and the level of animal rather than total fat in the diet. Thus the "pure" vegetarians, despite the free use of plant fats, but on diets devoid of animal fat, have the lowest serum cholesterol values. This is in accord with the recent observations of Kinsell and associates, [12,13] who found that subjects on diets containing large amounts of vegetable fat showed a marked decrease in serum cholesterol.

It has been pointed out by Moses[14] that "neither moderate increases or decreases in dietary cholesterol exert any consistently significant effect upon blood cholesterol levels." However, the duration of the dietary program may be a factor of importance. In most studies relating cholesterol content of diets and levels of serum cholesterol, subjects have pursued the experimental regimes for relatively short periods of time. In this investigation,

TABLE II

Comparison of Fat Intakes of Vegetarian and Non-vegetarian Groups

	L-o-vegetarian Group I			"Pure" vegetarian Group II			Non-vegetarian Group III			Comparison of groups: I & III		II & III		I & II	
	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	Diff. of Means	S.E. of Diff.	Diff. of Means	S.E. of Diff.	Diff. of Means	S.E. of Diff.
Per cent of Calories from Total Fat															
ADULT															
Males	15	33.1	7.11	14	35.2	10.07	15	43.0	5.35	9.9	2.30	7.8	3.40	2.1	3.40
Females	15	33.3	5.94	11	34.2	10.06	15	41.7	4.35	8.4	1.89	7.5	3.38	0.9	3.54
ADOLESCENT															
Males	15	34.0	4.31	1	51.3	—	15	41.3	2.51	7.3	1.77	—	—	—	—
Females	15	34.5	5.11	—	—	—	15	41.8	5.09	7.3	2.02	—	—	—	—
PREGNANT WOMEN	26	32.6	6.57	—	—	—	28	39.5	5.53	6.9	1.66	—	—	—	—
Per cent of Calories from Animal Fat															
ADULT															
Males	15	9.4	4.54	—	—	—	15	25.1	7.13	15.7	2.18	—	—	—	—
Females	15	13.1	5.53	—	—	—	15	23.9	6.69	10.8	2.24	—	—	—	—
ADOLESCENT															
Males	15	12.7	3.57	—	—	—	15	22.4	5.26	9.7	1.61	—	—	—	—
Females	15	13.2	4.22	—	—	—	15	25.2	6.07	12.0	1.91	—	—	—	—
PREGNANT WOMEN	26	13.8	4.98	—	—	—	28	26.0	6.27	12.2	1.54	—	—	—	—

TABLE III

Comparison of Serum Cholesterol Levels of Vegetarian and Non-vegetarian Groups

Groups	L-o-vegetarian			"Pure" vegetarian			Non-vegetarian			Comparison of groups							
										I & III			II & III			I & II	
	No.	Group I Cholesterol Mean	S.D.	No.	Group II Cholesterol Mean	S.D.	No.	Group III Cholesterol Mean	S.D.	Diff. of Means	S.E. of Diff.		Diff. of Means	S.E. of Diff.		Diff. of Means	S.E. of Diff.
		mg./100 ml.			*mg./100 ml.*			*mg./100 ml.*									
ADULT																	
Males	15	243	21.2	14	206	35.0	15	288	49.0	45	13.8		82	15.7		37	10.8
Females	15	269	61.0	11	206	32.7	15	295	62.8	26	22.6		89	19.0		63	18.6
ADOLESCENT																	
Males	15	194	26.7	—	—	—	15	214	35.0	20	11.6		—	—		—	—
Females	15	206	41.3	—	—	—	15	209	32.2	3	13.5		—	—		—	—
PREGNANT WOMEN	26	303	53.9	—	—	—	28	325	65.9	22	16.3		—	—		—	—

the lacto-ovo-vegetarians had maintained their dietary regimes of moderate cholesterol content throughout life, while the "pure" vegetarians had all consistently followed their cholesterol-free diets for a minimum of five or more years. Is it possible that the continued ingestion of exogenous cholesterol carried in animal fats over many years contributes to the gradual increase in serum cholesterol?

Because of the rarity of "pure" vegetarian subjects and the paucity of reliable clinical criteria, it was not possible to determine the incidence of atherosclerosis among the groups studied. Whether significant or not, it is interesting that no hypertensive subjects were observed among the "pure" vegetarians, although cases were encountered with equal frequency among the lacto-ovo-vegetarians and non-vegetarians.[15] The possible endogenous synthesis of cholesterol[16] from overgenerous caloric intake should be considered. The significantly lower body weight of the "pure" vegetarian men and women (average twenty pounds) as compared to the lacto-ovo-vegetarian and non-vegetarian adult groups may indicate, as suggested in an earlier paper,[15] that the efficiency of caloric utilization may be less in those subsisting on an all plant dietary than of those using foods of both animal and plant origin.

The possibility of a relationship between body weight and cholesterol level was examined. The subjects of each group were divided into those above or below their mean group weight, and those above or below their desirable weights. No correlation between weight and cholesterol concentration was found.

Keys *et al.*[17] have observed a relationship between serum cholesterol levels and age, where a gradual rise occurs in both sexes after the age of 30. Others[18,19] have reported similar findings. In Table IV, the subjects have been grouped according to age, being divided into those above or below mean group age. In general, the older subjects have measurably but not significantly higher cholesterol values. However, if the cholesterol levels of the lacto-ovo-vegetarian and non-vegetarian adoles-

TABLE IV
Comparison of Age and Cholesterol Values of Vegetarian and Non-vegetarian Groups

Groups	Subjects less than mean age			Subjects more than mean age			Comparison of groups	
	No.	Mean	S.D.	No.	Mean	S.D.	Diff. of Means	S.E. of Diff.
ADULTS								
L-o-vegetarian								
Males	7	238.9	24.95	8	246.5	20.0	7.6	11.8
Females	5	260.8	85.13	10	273.6	56.17	12.8	32.0
"Pure" vegetarian								
Males	7	190.4	19.5	7	222.6	31.2	32.2	15.0
Females	5	192.6	7.18	5	217.2	31.8	24.6	16.6
Non-vegetarian								
Males	9	275.9	53.21	6	313.2	48.07	37.3	26.5
Females	8	254.8	28.78	7	341.4	59.83	86.6	24.8

cents (Table III) are compared with the values for their respective adult groups, a significant increase in concentrations is seen.

Since the intake of dietary cholesterol of all groups of non-vegetarians was significantly greater than that of the corresponding lacto-ovo-vegetarian groups, it is possible that in the younger age groups (adolescents) of both sexes, where serum cholesterol levels are similar, the body has the ability to control or eliminate the excesses of both the endogenous or exogenous cholesterol, and thus maintain the serum cholesterol at a lower level. In the adult groups, however, the difference between the serum cholesterol concentrations of lacto-ovo-vegetarians and non-vegetarians is gradually widening. This might suggest that with advancing age the body's capacity to eliminate excess cholesterol, whether from endogenous or exogenous origin, diminishes, and that a gradual accumulation of cholesterol results.

Summary

The cholesterol intakes, as determined by calculation, and serum cholesterol measurements of a limited number of "pure" vegetarian, lacto-ovo-vegetarian, and non-vegetarian pregnant women, adolescents, and adults of both sexes are reported. The dietary cholesterol is higher in the non-vegetarian groups than in the lacto-ovo-vegetarian groups. The "pure" vegetarians' diet is, of course, cholesterol-free.

Serum cholesterol levels of the adult vegetarian groups tend to be lower than the adult non-vegetarian groups, the pure vegetarians having the lowest values. The significantly lower serum cholesterol concentration of the "pure" vegetarians occurred despite a free intake of vegetable fat. Cholesterol levels appear more closely correlated to the intake of animal fat than of total fat.

The higher serum cholesterol concentrations of the older adult groups as compared with the younger groups would support the observation of others that there occurs a gradual increase in cholesterol levels with advancing age. Since the differences in serum cholesterol in the adolescent age groups is only slight, it is suggested that with aging there may occur a diminishing ability of the body to handle excess cholesterol, whether of endogenous or exogenous origin.

Acknowledgment

The senior author wishes to express his appreciation to Mrs. Bertha S. Burke, Dr. Robert B. Reed, and Dr. George V. Mann for their help and advice during the course of this study.

REFERENCES

1. Okey, R.: Cholesterol content of foods. *J. Am. Dietet. A.* 21: 341, 1945.
2. Turner, D.: *Handbook of Diet Therapy.* University of Chicago Press, Chicago, Illinois, 1946.
3. Watt, B. K., and Merrill, A. L.: Composition of foods—raw, processed, prepared. *Agriculture Handbook*, No. 5, U. S. Department of Agriculture, 1950.
4. Bowes, A. D., and Church, C. F.: *Food Values of Portions Commonly Used.* Published by Senior Author, Philadelphia, 1951.

5. Bloor, W. R.: The determination of cholesterol. *J. Biol. Chem.* 24: 227, 1916.
6. Food and Agriculture Organization: *Food Balance Sheets.* Washington, D. C., 1949.
7. Phil, A.: Cholesterol studies. I The cholesterol content of food. *Scand. J. Clin. Lab. Invest.* 4: 115, 1952.
8. Keys, A., Chapman, L. B., Mickelsen, O., and Miller, E. v. O.: The relation in man between cholesterol levels in the diet and in the blood. *Science* 112: 79, 1950.
9. Mellinkoff, S. M., Machella, T. E., and Reinhold, J. G.: The effect of a fat-free diet in causing low serum cholesterol. *Am. J. Med. Sc.* 220: 203, 1950.
10. Morrison, L. M., Zwirlein, M., and Wolfson, E.: The effects of low fat—low cholesterol diets on the serum lipids. *Circulation* 2: 475, 1950.
11. Hildreth, E. A., Mellinkoff, S. M., Blair, G. W., and Hildreth, D. M.: The effect of vegetable fat ingestion on human serum cholesterol concentration. *Circulation* 3: 641, 1951.
12. Kinsell, L. W., Partridge, J., Boling, L., Sheldon, M., and Michaels, G.: Dietary modification of serum cholesterol and phospholipid levels. *J. Clin. Endocr. & Metab.* 12: 909, 1952.
13. Kinsell, L. W., Michaels, G. D., Partridge, J. W., Boling, L. A., Balch, H. E., and Cochrane, G. C.: Effect upon serum cholesterol and phospholipids of diets containing large amounts of vegetable fat. J. Clin. Nutrition 1: 224, 1953.
14. Moses, C.: Dietary cholesterol and atherosclerosis. *Am. J. Med. Sc.* 224: 212. 1952.
15. Hardinge, M. G., and Stare, F. J.: Nutritional studies of vegetarians. 1. Nutritional, physical, and laboratory studies. J. Clin. Nutrition 2: 73, 1954.
16. Block, K., and Rittenberg, D.: On the utilization of acetic acid for cholesterol formation. *J. Biol. Chem.* 145: 625, 1942.
17. Keys, A., Mickelsen, O., Miller, E. O., Hayes, E. R., and Todd, R. L.: The concentration of cholesterol in the blood serum of normal man and its relation to age. *J. Clin. Investigation* 29: 1347, 1950.
18. Sperry W. M., and Webb, M.: The effect of increasing age on serum cholesterol concentration. *J. Biol. Chem.* 187: 107, 1950.
19. Gertler, M. M., Garn, S. M., and Bland, E. F.: Age, serum cholesterol and coronary artery disease. *Circulation* 2: 517, 1950.

Resumen

Estudios nutricionales de vegetarianos. 2. Dieta y niveles de colesterol del suero

Se reporta la ingesta de colesterol determinada por medio de cálculos y medidas de colesterol del suero en un número limitado de mujeres encintas, adolescentes y adultos de ambos sexos, vegetarianos "puros," lacto-ovo-vegetarianos y no vegetarianos. El colesterol de la dieta es mayor en los grupos no vegetarianos que en los lacto-ovo-vegetarianos. La dieta de los vegetarianos "puros" es, por supuesto, libre del colesterol.

Los niveles de colesterol del suero tienden a ser menores en el grupo de adultos vegetarianos, encontrándose los valores menores en los vegetarianos "puros." Los niveles significativamente menores del colesterol del suero del grupo de vegetarianos "puros" ocurrieron a pesar de la ingesta libre de grasa vegetal. Los niveles de colesterol aparecen más directamente relacionados con la ingesta de grasa animal que con la de grasa total.

Las concentraciones más altas de colesterol del suero en el grupo de adultos mayores en comparación con el grupo de jóvenes puede soportar la observación realizada por otros autores de que ocurre un aumento gradual de los niveles de colesterol conforme avanza la edad. Desde que las diferencias en el colesterol del suero en los grupos de adolescentes es solamente ligera, se sugiere que con la edad puede ocurrir una disminución de la habilidad del organismo para "manejar" el exceso de colesterol, ya sea de origen endógeno o exógeno.

Correction

In Vol. 1 (7), page 534, column 2, line 3, for 0.1, read 0.001.

Serum and Urine CONCENTRATIONS *of* VITAMIN B_{12} *following Oral Administration of the Vitamin*

By W. R. PITNEY, M.D.* AND M. F. BEARD, M.D.†

SMALL amounts of vitamin B_{12}, of the order of 5 micrograms (μg.), when given daily by mouth to patients with pernicious anemia in relapse will not cause hematological improvement.[1,2] When these same amounts of the vitamin are administered along with neutralized normal gastric juice, a hematological remission can be produced.[1,3,4] These findings infer that small amounts of vitamin B_{12} can be absorbed from the intestine in pernicious anemia in the presence of normal gastric juice. Studies using radioactive (Co^{60}) vitamin B_{12} confirm this presumption. When a tracer dose of radioactive vitamin B_{12} is given orally to a patient with pernicious anemia in relapse, most of the radioactivity can be recovered from the feces. The simultaneous administration of hog stomach concentrate or normal gastric juice materially reduces the fecal excretion, providing evidence that at least part of the dose has been absorbed.[5-7] A recent test for intrinsic factor activity[8] has provided more direct evidence that small amounts of radioactive vitamin B_{12} are absorbed from the intestine in normal individuals, but not in patients with pernicious anemia, even when in remission. If a large parenteral dose of non-radioactive vitamin is given two hours after a small oral dose of radioactive vitamin B_{12}, radioactivity appears in the urine of normal individuals, but not in cases of pernicious anemia, unless normal gastric juice is administered concurrently with the oral dose.

However, very large doses of vitamin B_{12} when given orally to patients with pernicious anemia in relapse, can bring about hematological improvement, even without the concurrent administration of normal gastric juice.[2,9,10,11,12] Remission has followed when the vitamin has been given as a single large dose, or when administered in daily doses. It has been previously noted that following these large oral doses, urinary excretion of the vitamin is minimal,[9,11] whereas there is appreciable excretion of the vitamin following the parenteral injection of much smaller doses. Conley *et al.*[9] reported complete hematological remission following a single oral dose of 5000 μg. On the basis of the amount of vitamin B_{12} given parenterally which is needed to produce this effect, they calculated that at least 25 μg. of the oral dose was absorbed. Following the parenteral injection of amounts of vitamin B_{12} of 40 μg. or greater, there is a prompt increase in the vitamin B_{12} activity of

From the Division of Hematology, University of Louisville School of Medicine.

* Research Fellow in Hematology, University of Louisville School of Medicine, Louisville, Kentucky.

† Director, Division of Hematology; Associate Clinical Professor of Medicine, University of Louisville School of Medicine.

This study was made possible by a grant from the National Institutes of Health, Number A-112 (C2S), Bethesda, Maryland.

Vitamin B_{12} used in this study furnished by E. R. Squibb & Sons, New York, N. Y.

The technical assistance of Mrs. Frances Jones is gratefully acknowledged.

the urine.[13,14] However, Conley *et al.* could find no measurable increase in urinary activity following oral doses as high as 10,000 μg. These observations suggest either (a) that at no time following oral ingestion of these large doses by patients with pernicious anemia do the serum levels of vitamin B_{12} rise as high as they do following parenteral injections of the order described, or (b) that vitamin B_{12} is absorbed from the intestine in a form not readily excreted by the kidneys, whereas a considerable amount of injected vitamin B_{12} can be excreted.

Vitamin B_{12} is normally present in the serum as a heat labile complex with serum protein.[15] The binding capacity of serum for vitamin B_{12} is limited.[19] It has been shown that following parenteral injection of vitamin B_{12} to patients with pernicious anemia in relapse, there is an increase in both the bound and free form of the vitamin in serum.[13] Urinary excretion of the vitamin occurs during the period of time that there are demonstrable levels of free vitamin in the serum. Apparently *free* vitamin B_{12}, but not vitamin B_{12} *bound* to serum protein, is excreted by the kidneys.

A possible explanation of the failure to detect urinary excretion following oral dosage with large amounts of vitamin B_{12} could be that the rises in serum concentration following such therapy represent vitamin B_{12} in bound form, and that levels of free vitamin do not occur to the same extent as following parenteral therapy. In this paper are recorded the serum and urinary concentrations of the vitamin following oral therapy to six cases of pernicious anemia in relapse. As a comparative study, similar quantities of vitamin B_{12} were administered orally to four normal individuals.

Material and Methods

Vitamin B_{12} concentrations of serum and urine were estimated microbiologically, using *Euglena gracilis* as test organism. The technique was exactly that described by Ross.[16] This method of assay is able to distinguish between free and bound forms of the vitamin in serum. In our laboratory, the concentration of vitamin B_{12} found in 56 normal sera shows a range from 86–460 $\mu\mu$g./ml., with a mean of 212 $\mu\mu$g./ml. In 52 of the sera, the vitamin was estimated to be totally in the bound form. In the other four, small amounts of free vitamin were found. The mean bound concentration for the whole group is 207 $\mu\mu$g./ml. The patients with pernicious anemia studied fulfilled the following criteria for diagnosis: macrocytic anemia, megaloblastic bone marrow, histamine-fast achlorhydria, and a very low or absent concentration of vitamin B_{12} in the serum.

Most patients underwent an initial control period, during which at least two samples of blood were withdrawn on successive days for estimation of serum vitamin B_{12} concentration, and a 24-hour urine collection was made. Vitamin B_{12} was administered in a single dose with water early in the morning after an overnight fast. No food was allowed for one hour after the dose had been administered. Samples of venous blood were obtained 30 minutes, 1, 2, 4, 6, 12, and 24 hours after the oral dose, and then daily until such time as parenteral therapy was instituted. Urine was collected for 24 hours following the dose. Urine volumes were measured, and aliquots frozen at −20° C. along with the samples of sera until they could be assayed as a single batch. Experiments on the normal individuals were similar, except that observations were discontinued 24 hours after the oral administration of the vitamin.

Results

1. *Serum Concentrations of Vitamin B_{12} in the First 24 Hours following Oral Therapy to Pernicious Anemia Patients*

The results of the serum assays in the six patients with pernicious anemia in relapse are shown in Table I. No vitamin B_{12} activity was detected in four cases (cases 1, 2, 5, 6) before therapy. In case 3, the serum concentration was 20 $\mu\mu$g./ml.; in case 4, 16 $\mu\mu$g./ml. As stated above, normal sera have not assayed below 86 $\mu\mu$g./ml. in our laboratory. The vitamin present in normal serum is nearly all in the bound form.

Two patients were given an oral dose of 1000 μg. (cases 1 and 2). In case 1, no de-

TABLE I

Serum Concentrations of Vitamin B_{12} ($\mu\mu$g./ml.) in the First 24 Hours following Oral Therapy to Pernicious Anemia Patients

Case no.	Dose	0 hr.	1/2 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.
	μg.								
1	1000	0	0	0	0	0	0	0	0
2	1000	0	—	16	16	20	16	0	0
3	3000	20	—	—	66	100	—	—	96
4	5000	16	—	109	285	210	231	—	184
5	5000	0	—	167	—	289	235	—	131
6	9000	0	152	—	170	152	165	—	83

tectable rise in serum concentration of vitamin B_{12} was noted. In case 2, there was a slight rise in concentration to a maximum of 20 $\mu\mu$g./ml. shown in the four-hour specimen. No demonstrable vitamin B_{12} activity was detected after 24 hours. Case 3 was given 3000 μg. orally. It was not possible to collect frequent venous samples from this patient. There was a rise in serum concentration from 20 $\mu\mu$g./ml. to 100 $\mu\mu$g./ml. in the four-hour specimen. Twenty-four hours after the dose was given, the serum concentration was 96 $\mu\mu$g./ml. In all samples, the vitamin B_{12} present was totally in the bound form.

Two patients (cases 4 and 5) were given a single oral dose of 5000 μg. In both, a rise in serum concentration to the normal range was seen within one hour. Twenty-four hours later, serum concentrations were still within the normal range. In case 4, there was a trace only of free vitamin (less than 40 $\mu\mu$g./ml.) in specimens drawn at 2, 4, and 6 hours. The remainder of the vitamin in these specimens, and all the vitamin in the one-hour and 24-hour specimens, was estimated to be in the bound form. In case 5, free vitamin was detected up to six hours. There was 50 $\mu\mu$g./ml. free vitamin in the one-hour sample, 80$\mu\mu$g./ml. at 4 hours, and 100 $\mu\mu$g./ml. at 6 hours. In the 24-hour specimen, the vitamin present was assayed as totally bound.

Case 6 was given 9000 μg. orally. A blood specimen withdrawn 30 minutes later revealed a serum concentration of vitamin B_{12} already in the normal range. The serum concentration remained normal for 24 hours. None of these specimens revealed the presence of free vitamin.

TABLE II

24-Hour Urinary Excretion of Vitamin B_{12} (μg.) in Cases of Pernicious Anemia before and after Oral Therapy

Case no.	Oral dose	24-hr. control period	24-hr. period after therapy
	μg.	μg.	μg.
1	1000	0	0
2	1000	—	—
3	3000	—	0.134
4	5000	0	0.147
5	5000	0	0.273
6	9000	0	0.45

2. Urinary Excretion of Vitamin B_{12} following Oral Therapy to Pernicious Anemia Patients

The urinary excretion of vitamin B_{12} for the 24-hour control period prior to oral therapy, and for the succeeding 24 hours is shown in Table II. In only 4 cases was it possible to assay control urine specimens. In none of these was any vitamin B_{12} activity demonstrated. The greatest amount of vitamin B_{12} excreted following the dose was 0.45 μg. This occurred following the administration of 9000 μg. vitamin B_{12} orally. The post-therapy excretion figures for cases 3–6 are probably within the range of excretion for normal individuals on a normal diet.[17]

3. Daily Observations on the Serum Concentration of Vitamin B_{12} following Single Oral Dosage to Pernicious Anemia Patients

Frequent, usually daily, serum vitamin B_{12} assays were performed on these six patients subsequent to the oral therapy. Observations were continued until such time as it was de-

TABLE III

Serum Concentrations of Vitamin B_{12} ($\mu\mu$g./ml.) Estimated Daily, following Single Oral Therapy to Pernicious Anemia Patients

Day	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Pre-therapy	0	0	20	16	0	0
1	0	0	96	184	131	83
2	0	0	73	120	87	60
3	0	0	—	84	—	—
4		—	62	97	73	—
5		0	—	134	67	50
6		0	61	124	57	48
7		0		128	58	38
8				—	41	40
9				112	48	33
10				—	—	42
11				114	21	—
12					23	42
13					18	
14					18	
15					30	
19					25	
21					25	
23					25	
25					23	

cided to institute parenteral therapy. The results are shown in Table III. In cases 3 to 6, where a rise in serum concentration was achieved, vitamin B_{12} activity could be detected in the serum for several days. With the exception of case 4, the values obtained were below the range seen in normal individuals. In every specimen, vitamin B_{12} was present exclusively in the bound form. Case 5 was studied for 25 days following therapy. At the end of this time, some vitamin B_{12} activity could still be detected in the serum, but the concentration was very low.

4. Hematological Response following Oral Therapy

It is not the purpose of this paper to supply complete hematological data on these patients. Detailed blood counts were performed daily where possible. In some cases, the bone marrow was examined at intervals during the period of observation. As soon as it was felt that hematological improvement had ceased to occur in those cases in which it was noted, patients were given an intramuscular injection of 30 μg. crystalline vitamin B_{12}. This dose has been repeated every two weeks. In the first two cases, the experiment had to be discontinued because of anxiety over the general condition of the patient.

Case Reports

Case 1 was a 46-year-old colored female, whose blood examination showed a red cell count of 1.19 million/c.mm.; hemoglobin, 3.2 grams per cent; PCV (packed cell volume), 13%; and reticulocytes, 8.4%. She was severely ill and disorientated and was observed for three days following an oral dose of 1000 μg. crystalline vitamin B_{12}. During this time, the PCV fell to 10.5%, the reticulocyte count remained moderately elevated, and her general condition did not improve. Another bone marrow examination on the third day again showed megaloblastic erythropoiesis. The experiment was then abandoned and she was given parenteral therapy with 40 μg. vitamin B_{12}. The hematological response was satisfactory, a reticulocytosis of 34% being achieved four days after the parenteral injection and seven days after the oral dose. Her subsequent course has been uneventful.

Case 2 was a 58-year-old colored male. The red cell count was 1.62 million/c.mm.; hemoglobin, 4.9 grams per cent; PCV, 15.5%; and reticulocytes, 0.6%. He was observed for seven days following oral therapy with 1000 μg. vitamin B_{12}. A reticulocyte count of 6% was seen on the sixth day, which, however, had dropped to 4% the next day. There was no change in the red cell count and PCV. He was also quite ill and on the seventh day was given 30 μg. vitamin B_{12} parenterally. A reticulocyte peak of 17% was seen five days later. His subsequent course has been uneventful.

Case 3 was a 70-year-old white male. He was not a hospital patient and it was not possible to do sufficiently frequent blood counts to draw conclusions as to the effectiveness of oral therapy. He was treated parenterally on the sixth day and referred to his private physician.

Case 4 was a 76-year-old white male, who was observed for 11 days after an oral dose of 5000 μg. vitamin B_{12}. Initially, his blood showed a red cell count of 2.47 million/c.mm.; hemoglobin, 7.6 grams per cent; PCV, 25%; and reticulocytes, 0.2%. A reticulocytosis of 7.4% was noted after four days, and another bone marrow examination on this day showed normoblastic erythropoiesis. By the 11th day the PCV had risen to 32%. He was then given parenteral therapy, and his subsequent course has been uneventful.

Case 5 was an 86-year-old white male. He was observed for 25 days following an oral dose of 5000 μg. vitamin B_{12}. The red cell count was 1.17 million/c.mm.; hemoglobin, 4.8 grams per cent; PCV 19%;

and reticulocytes, 0.8%. A reticulocyte peak of 17.6% was noted five days after the oral dose. The PCV subsequently rose to 33% by the 21st day, but then leveled off, and four days later was 32%. A further bone marrow examination at this time again showed megaloblastic erythropoiesis. He was therefore given parenteral therapy, and his subsequent course has been uneventful.

Case 6 was a 75-year-old white female whose initial blood count showed 1.72 million red cells/c.mm.; hemoglobin, 7.5 grams per cent, PCV, 21%; and reticulocytes, 0.1%. Five days after the oral dose of 9000 μg. vitamin B_{12}, a reticulocytosis of 10% was observed. She was followed for a total of 12 days. At the end of this time, the PCV had risen to 28%. She was then given parenteral therapy, and her subsequent course has been uneventful.

5. Serum Concentrations of Vitamin B_{12} in the First 24 Hours following Oral Dosage to Normal Individuals

In four normal young adult males, serum concentrations of vitamin B_{12} were determined at frequent intervals for 24 hours following oral dosage. Two subjects were given 1000 μg. vitamin B_{12} and the other two 5000 μg. The results of the serum assays are shown in Table IV. In each case, the pretreatment serum concentration was in the normal range. Following 1000 μg. orally, there was a rise in serum concentration in one subject from 184 $\mu\mu$g./ml. to a maximum of 216 $\mu\mu$g./ml. at two hours; in the other individual, the concentration rose from 272 $\mu\mu$g./ml. to a maximum of 324 $\mu\mu$g./ml. at four hours. These rises have doubtful significance when normal fluctuations of concentration and the accuracy of the assay technique are considered. The vitamin B_{12} in all specimens was exculsively in the bound form.

When 5000 μg. was given orally, significant rises in serum concentration were observed in normals. In one case, a rise from 168 $\mu\mu$g./ml. to a maximum of 344 $\mu\mu$g./ml. at the end of six hours was noted; in the other, a rise from 236 $\mu\mu$g./ml. to a maximum of 620 $\mu\mu$g./ml. at the end of two hours. Again, no free vitamin B_{12} was detected in any of these sera.

6. Urinary Excretion of Vitamin B_{12} in Normals following Oral Dosage

In Table V are shown the urinary excretions of vitamin B_{12} in these four normals for the 24-hour period immediately prior to the oral dose, and for the succeeding 24 hours. In each case, there was a rise in urinary excretion over the control amounts, but the increase was of the same order as was seen with the pernicious anemia patients (Table II).

TABLE IV

Serum Concentrations of Vitamin B_{12} ($\mu\mu$g./ml.) in 4 Normal Individuals following Oral Dosage

	Dose given	Control Serum conc.	1/2 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.
	μg.								
1	1000	184	184	180	216	200	184	164	220
2	1000	272	232	244	288	324	232	212	216
3	5000	168	168	236	228	304	344	292	240
4	5000	236	280	372	620	580	432	—	416

Discussion

We have assayed sera for vitamin B_{12} concentration in seven additional cases of pernicious anemia in relapse, as well as the six cases investigated in this paper. In the total group of 13, eight revealed *no* vitamin B_{12} activity. In the remaining five, concentrations from 16–44 $\mu\mu$g./ml. were found. These findings confirm other reports[11,17] that there is a reduction in the vitamin B_{12} concentration of the serum in pernicious anemia. The value of

TABLE V

Urinary Excretion of Vitamin B_{12} in Normals following Oral Dosage

	Oral dose	24-hr. control period	24-hr. period after therapy
	μg.	μg.	μg.
1	1000	0.142	0.217
2	1000	0.103	0.208
3	5000	0.146	0.505
4	5000	0.134	0.420

this test in determining which therapeutic agent to employ in the treatment of megaloblastic anemia has been pointed out before.[18]

In those cases treated with oral vitamin B_{12}, a correlation has been observed between changes in serum concentration of the vitamin and subsequent hematological improvement. In case 1, serum and urine assays did not show any evidence of absorption from the 1000 μg. dose, and the bone marrow remained megaloblastic. The slight transient rise in serum concentration in case 2 after a similar oral dose may account for the reticulocytosis of 6 per cent noted on the sixth day, but clinical evidence that the absorption was suboptimal is supplied by the failure to observe a rise in the red cell count, and the appearance of a second reticulocytosis when parenteral therapy was instituted. Cases 4, 5, and 6 all showed a hematological response, and in all, significant rises in serum concentrations of vitamin B_{12} were achieved after oral therapy. In case 5, the bone marrow had reverted to megaloblastic erythropoiesis while the serum concentration was still about 20 $\mu\mu$g./ml. This suggests that the critical level of serum concentration to maintain normal erythropoiesis is above this figure.

The fact that absorption of vitamin B_{12} *does* occur in patients with pernicious anemia in relapse following massive oral therapy suggested to Meyer *et al.*[2] that the deficiency in intrinsic factor may not always be complete. More probable, however, is the supposition that absorption following these large doses represents a flooding phenomenon; such high concentrations are achieved within the lumen of the gut that vitamin B_{12} is passively transferred through the mucosal surface. That a physiological process involving intrinsic factor is not involved is suggested by the observation that the pattern of response in the serum concentration of the vitamin is similar in pernicious anemia patients and normal individuals.

It is probable that the rate or manner of absorption from the intestine is an important factor in determining the amount of vitamin excreted in the urine. Immediately after a parenteral injection of crystalline vitamin B_{12}, the circulation is flooded with the free vitamin. Some of this is bound by serum protein, but the capacity of the serum to bind vitamin B_{12} is limited.[19] Probably no more than 1 μg. can be disposed of in this way.[13] An unknown percentage of the remainder can be fixed in tissue storage depots, but this is not an immediate process, and meanwhile the free vitamin can escape through the kidneys. In the oral absorption experiments reported in this paper, the free vitamin was detected in the serum in only two cases. These were both patients with pernicious anemia. In case 4, the amount of free vitamin detected was less than 40 $\mu\mu$g./ml. In case 5, 100 $\mu\mu$g./ml. of free vitamin was detected in the six-hour specimen. It is, however, not unusual to find small amounts of free vitamin in the serum of normal individuals,[17] and the highest level reported in these experiments is much lower than that usually found after parenteral therapy.

It is difficult to explain why normal individuals do not show rises in serum concentration after an oral dose of 1000 μg. vitamin B_{12}. This observation has been previously described.[20] Experiments with radioactive vitamin B_{12} have demonstrated absorption in normals from very small oral doses of the vitamin. Vitamin B_{12} absorbed from the intestine must pass through the liver before appearing in the general circulation. If absorption is a slow process, it is possible that the liver may be able to store the extra vitamin as it is presented to the organ, so that concentrations remain relatively constant in the general circulation. However, direct assays on portal blood in dogs after duodenal instillation of the vitamin, have been reported[21] and they do not differ from those found in peripheral venous blood.

Summary

In both normal individuals and patients with pernicious anemia in relapse, serum concentrations of vitamin B_{12} show *no* significant alteration following the oral administration of 1000 μg. of the vitamin. When the dosage is increased to 5000 μg., serum concentrations in both groups are increased. In pernicious

anemia, there is a correlation between hematological response and the demonstration of the vitamin in the serum following oral therapy. Vitamin B_{12} appears in the serum in combined form after adequate oral dosage. The absence of circulating free vitamin accounts for the failure to detect the increased urinary activity which is observed following parenteral therapy.

Addendum

Since this paper was received for publication, Unglaub and associates[22] have reported similar observations concerning the oral absorption of vitamin B_{12}, using a modified *L. leichmannii* assay method for the determination of serum concentration. Normal subjects did not show an appreciable rise in serum concentration after oral doses less than 3000 μg. Five out of six pernicious anemia patients showed a marked rise in concentration after 3000 μg. orally. Their assay method does not distinguish free from bound forms of the vitamin in serum; however, they postulated that vitamin B_{12} may be absorbed from the intestine in bound form. This we have shown to be true. It is not practical, however, to estimate the quantity of vitamin absorbed after an oral dose by comparing the urinary excretion with that found after smaller intramuscular injections, because the vitamin appears in the serum in different forms after these different methods of administration.

REFERENCES

1. Hall, B. E., Morgan, E. H., and Campbell, D. C.: Oral administration of vitamin B_{12} in pernicious anemia, I. Presence of intrinsic factor in Berkefeld—filtered pooled human gastric juice: Preliminary report. *Proc. Staff Meet., Mayo Clin.* 24: 99, 1949.
2. Meyer, L. M., Sawitsky, A., Cohen, B. S., Krim, M., and Fadem, R.: Oral treatment of pernicious anemia with vitamin B_{12}. *Am. J. Med. Sc.* 220: 604, 1950.
3. Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R., and Epstein M.: Observations on the etiological relationship of achylia gastrica to pernicious anemia. X. Activity of vitamin B_{12} as food (extrinsic) factor. *New England J. Med.* 239: 911, 1948.
4. Schilling, R. F., Harris, J. W., and Castle, W. B.: Observations on the etiological relationship of achylia gastrica to pernicious anemia. XIII. Hematopoietic activity of vitamin B_{12a} (vitamin B_{12b}). *Blood* 6: 228, 1951.
5. Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H.: Studies of excretion (and absorption) of Co^{60} labeled vitamin B_{12} in pernicious anemia. *Tr. A. Am. Physicians* 65: 214, 1952.
6. Meyer, L. M.: Oral absorption of Co^{60} vitamin B_{12} in pernicious anemia. *Proc. Soc. Exp. Biol. & Med.* 82: 490, 1953.
7. Meyer, L. M., Becerra-Garcia, H., Goldman, A., and Stern, P. A.: Oral administration of Co^{60} vitamin B_{12} to normal persons, patients with pernicious anemia and subjects with various medical disorders. *J. Applied Physiol.* 6: 263, 1953.
8. Schilling, R. F.: Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12}. *J. Lab. & Clin. Med.* 42: 860, 1953.
9. Conley, C. L., Krevans, J. R., Chow, B. F., Barrows, C., and Lang, C. A.: Observations on the absorption, utilization and excretion of vitamin B_{12}. *J. Lab. & Clin. Med.* 38: 84, 1951.
10. Pitney, W. R., and Beard, M. F.: Serum vitamin B_{12} concentrations in pernicious anemia (Abst.). *J. Lab. & Clin. Med.* 42: 928, 1953.
11. Unglaub, W. G., Rosenthal, H. L., and Goldsmith, G. A.: Vitamin B_{12} in blood and urine after oral and parenteral administration. *Fed. Proc.* 12: 432, 1953.
12. Ungley, C. C.: Absorption of vitamin B_{12} in pernicious anemia. I. Oral absorption without a source of intrinsic factor. *Brit. M. J.* 2: 905, 1950.
13. Mollin, D. L., and Ross, G. I. M.: Vitamin B_{12} concentrations of serum and urine in the first 72 hours after intramuscular injections of the vitamin. *J. Clin. Path.* 6: 54, 1953.
14. Sokoloff, M. F., Sanneman, E. H., and Beard, M. F.: Urinary excretion of vitamin B_{12}. *Blood* 7: 243, 1952.
15. Ross, G. I. M.: Vitamin B_{12} assay in body fluids. *Nature* 166: 270, 1950.
16. Ross, G. I. M.: Vitamin B_{12} assay in body fluids using euglena gracilis. *J. Clin. Path.* 5: 250, 1952.
17. Mollin, D. L., and Ross, G. I. M.: The vitamin B_{12} concentrations of serum and urine of normals and of patients with megaloblastic anemias and other diseases. *J. Clin. Path.* 5: 129, 1952.
18. Mollin, D. L., and Ross, G. I. M.: Vitamin B_{12} in practice. *Lancet* 1: 46, 1953.
19. Pitney, W. R., Beard, M. F., and VanLoon, E. J.: Observations on the bound form of vitamin B_{12} in human serum. *J. Biol. Chem.* 207: 143, 1954.
20. Rosenthal, H. L., and Sarett, H. P.: The determination of vitamin B_{12} activity in human serum. *J. Biol. Chem.* 199: 433, 1952.

21. Rosenthal, H. L., and Hampton, J. K.: Absorption of vitamin B_{12} in dogs. *Fed. Proc.* 12: 428, 1953.

22. Unglaub, W. G., Rosenthal, H. L., and Goldsmith, G. A.: Studies of vitamin B_{12} in serum and urine following oral and parenteral administration. *J. Lab. & Clin. Med.* 43: 143, 1954.

Resumen

Concentraciones de vitamina B_{12} en la orina y en el suero siguiendo la administración oral d esta substancia

Tanto en indivíduos normales como en pacientes con anemia perniciosa en recaída, las concentraciones séricas de vitamina B_{12} no muestran alteración significante a continuación de la administración oral de 1000 microgramos de la vitamina. Cuando la dosis se aumenta a 5000 microgramos, las concentraciones del suero aumentan en ambos grupos. En la anemia perniciosa existe una correlación entre la respuesta hematológica y la demonstración de la vitamina en el suero a continuación de la terapia oral. La vitamina B_{12} aparece en el suero en forma combinada a continuación de una terapia oral adecuada. La ausencia de vitamina circulante en forma libre explica el fracaso para demostrar al aumento de la actividad urinaria que se observa a continuación de la terapia parenteral.

A Gourmet on Obesity

"Obesity has an unfortunate influence on both sexes, in that it injures strength and beauty.

"It injures strength because, in augmenting the weight of the mass to be moved, it does not augment the motive power; it injures it further in interfering with respiration, which makes impossible all work which demands the prolonged use of muscular strength.

"Obesity injures beauty in destroying the harmony of proportion originally established; because all the parts do not grow larger to an equal degree . . . nothing is so common as to come upon physiognomies once very piquant and which obesity has rendered almost insignificant.

"It also predisposes to various diseases, such as apoplexy, dropsy, ulcers of the legs, and makes all the other illnesses more difficult to cure."

—Anthelme Brillat-Savarin (1755–1826) in *La Physiologie du Goût.*

Leanness and the Ladies

"Leanness is not a great disadvantage for men; they have on its account no less vigor, and are much more agile.

"But it is a horrible misfortune for women; since for them beauty is more than life, and beauty consists above all in the roundness of forms and the gracious curvature of lines. The most careful *toilette,* the most sublime *couturière* cannot mask certain absences, nor dissimulate certain angles; and it is commonly said that for every pin she removes, a thin woman, however beautiful she may appear, loses something of her charms.

"With the puny ones there is no remedy, or rather it is for the Faculty to concern themselves with such, and the regimen may be so long that the cure will arrive very late indeed.

"But for women who are born thin and who have a good stomach, we see no reason why they should be any harder to fatten than pullets; and if a little more time is necessary, it is because women have a comparatively smaller stomach, and cannot be put on a rigorously and punctually executed regimen like these devoted animals.

"This comparison is the most gentle one I have been able to find; I had to have one, and the ladies will forgive it, on account of the laudable intentions with which the chapter was conceived."

—Anthelme Brillat-Savarin (1755–1826) in *La Physiologie du Goût.*

The Effect of VITAMIN SUPPLEMENTATION *on* SOLDIERS *Residing in a* COLD ENVIRONMENT

PART I. PHYSICAL PERFORMANCE AND RESPONSE TO COLD EXPOSURE

By ROBERT RYER III, LT. COL., MSC, MORTON I. GROSSMAN, CAPT., MC, THEODORE E. FRIEDEMANN, PH.D., WILLIAM R. BEST, 1ST LT., MC, C. FRANK CONSOLAZIO, W. JAMES KUHL, 1ST LT., MC, WILLIAM INSULL, JR., 1ST LT., MC, FREDERICK T. HATCH, CAPT., MC, AND THE STAFF OF THE U. S. ARMY MEDICAL NUTRITION LABORATORY

IN RECENT years the concept has arisen that when severe demands are placed upon animal or human subjects the ability to withstand these demands may, in some instances, be improved by the administration of very large amounts of certain vitamins. A field experiment was conducted at Pole Mountain, Wyo., during the period January 2, 1953 to March 10, 1953 to test certain aspects of this hypothesis.

It was recognized at the outset that the variety of vitamin supplements and the diversity of environmental stresses which had been studied under laboratory conditions were so great that certain limited objectives would have to be sought in this study. The major points that had to be decided upon in designing the study were these: (1) the kind and amount of vitamins to be administered; (2) the types of stress to be imposed; and (3) the measures of response to stress to be used. The vitamin supplement used was a mixture of ascorbic acid and members of the B-complex; the stresses imposed consisted of high physical activity, cold exposure, and caloric restriction; and the measures of response to stress consisted primarily of tests of physical performance, with certain ancillary biochemical determinations.

The basic question which this study was designed to answer was: "Will the functional abilities of the soldier in a cold environment be significantly bettered by supplementation with large amounts of vitamin C and B-complex?"

A detailed report of this study has been published as Report Number 115 of the Medical Nutrition Laboratory, September 15, 1953. This paper will describe only the salient features of the experiment; the reader is referred to the report for detailed information and a review of the literature.

TEST SUBJECTS

The test subjects were 83 enlisted men and 4 officers, all of whom volunteered to serve as test subjects. The enlisted men were from Beaumont Army Hospital, Fort Bliss, Tex.

These men had, for the most part, been employed at Beaumont Army Hospital in various technical capacities, such as dental, surgical, medical laboratory, and pharmacy technicians. Some of the higher ranking noncommissioned officers were in administrative and supervisory positions.

The test subjects were formed into a company of four platoons. The most experienced

From the U. S. Army Medical Nutrition Laboratory, Denver, Colo.

The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

noncommissioned officers were designated platoon sergeants and squad leaders. The balance of the company was organized into four platoons of three squads each to produce platoons of as nearly equal numerical strength as possible. These assignments were made entirely on the basis of an alphabetical roster of the men, without regard to size, ability, or past performance of duty, in order that each platoon, exclusive of its noncommissioned officers in charge, might represent a fair cross-section of the overall composition of the company.

The four commissioned platoon leaders of the test company, who were also test subjects, volunteered directly from the student body at the Medical Field Service School, Brooke Army Medical Center, Ft. Sam Houston, Tex.

Location of Test Camp

The test was conducted at a camp at Pole Mountain, Wyo., located approximately five miles northeast of the Lincoln Highway (Route 30), in Medicine Bow National Forest. The elevation of the campsite was 8300 feet.

Operation of the Test

GENERAL PLAN

The study was divided into five phases which are shown in Table I. During the first two weeks, the first phase, the activity of the men was progressively increased to a level of approximately 4000 calories, while they adjusted themselves to the 4000 calorie diet, the clothing, the increased level of activity, the cold environment, and the altitude. During the second phase, which lasted three weeks, the caloric expenditure and the diet were maintained at the level of approximately 4000 calories. The sixth week, the third phase of the experiment, was a week without prescribed activity, except for certain testing procedures, and a continuation of the 4000 calorie diet; it constituted a week of rest for the test subjects. During the fourth phase of the study, weeks 7, 8, and 9, the caloric intake of the subjects was restricted to 2500 calories initially, then further reduced to approximately 2000 calories, while the energy expenditure of the men was maintained at the level of approximately 4000 calories. During the last phase of the study, the tenth week, the men rested; they had no prescribed activity other than certain test procedures and were fed the A-Ration in unrestricted amounts.

TABLE I
Phases of the Experiment

Phase	Week	Calories in diet
1. Equilibration	1 2	4000
2. High activity	3 4 5	4000
3. Rest and rehabilitation	6	4000
4. High activity	7	2500
	8	2500 to 2000
	9	2000
5. Rest and rehabilitation	10	Unrestricted "A" ration

TABLE II
Time Table of Dietary Regimes

Designation of dietary periods	Dates of dietary periods, 1953	Length of time, weeks	Calories offered per man per day, avg.
Equilibration	Jan. 5–18	2	4138
High calorie	Jan. 19–Feb. 8	3	4138
Rest and recovery	Feb. 9–15	1	4015
Low calorie, initial	Feb. 16–26	1.6	2569
Low calorie, final	Feb. 27–Mar. 10	1.4	2056

DIETARY REGIMES

The dietary regimes can conveniently be divided into five dietary periods. The caloric content and period of use of each regime are listed in Table II. The composition of the diets in terms of major dietary constituents for each dietary period are given in Table III.

Menus

During each of the five dietary periods four menus were used. Menus 1, 2, and 3 were repeated in rotation twice weekly and Menu 4 was served on Sunday. For each dietary period the calories, protein, fat, carbohydrate, and ascorbic acid were approximately the same in all four menus. Each menu provided three meals (breakfast, lunch, and dinner) and an evening snack.

TABLE III
Major Dietary Constituents in Diets of Each Period

Periods	Grams	Calories	Protein	Fat	Carbohydrate	Ascorbic acid
			Gm.	*Gm.*	*Gm.*	*mg.*
Equilibration	2617	4138	151	178	502	39.6
High calorie	2617	4138	151	178	502	39.6
Rest and recovery	2620	4015	144	184	464	36.0
Low calorie (2500)	1839	2569	78	95	354	43.3
Low calorie (2000)	1521	2056	74	77	271	38.5

Type of Foods Used in Each Dietary Period
The foods used in the equilibration and high calorie periods included (1) items from several of the packaged rations, particularly the C-Ration and the In-Flight Food Packet, (2) other canned fruits and vegetables and dry cereals, and (3) fresh items including white bread (unenriched), butter, and whole milk.

The same menus, except for meat items, were used in the rest and rehabilitation period (week 6) as in the equilibration and high calorie periods. Canned meats were deleted from the dietary and fresh meat was substituted. The quantity and type of meat remained the same. The amount per serving was adjusted to give the same protein content as the canned meat.

In the 2500 and 2000 calorie dietaries the menu pattern was the same as in the high calorie period; the only difference was in the restriction of calories and protein and the continued substitution of fresh meat for most of the C-Ration canned meat items as in the rest and rehabilitation week.

Control of Test Subjects' Food Intake

The test subjects did not have access to any food except that served in the test mess. They were instructed not to bring food with them in their luggage and an inspection was carried out soon after their arrival at the camp site to ascertain that there had been compliance with this order. During the entire period of the experiment there was no store or PX accessible to the subjects at which they could purchase food. The orders which the men placed for PX items excluded food. All packages received by mail by the test subjects were inspected by an officer and were given to the subjects only after it had been shown that they contained no food. The cooks and other food handlers were carefully and repeatedly instructed not to give extra food to test subjects and not to leave any food where test subjects might take it. Test subjects were instructed not to exchange food in the mess hall and not to take food from the mess hall. Observers were stationed in the mess hall to assure compliance with these regulations.

Test subjects working as dining room orderlies as part of the work detail were restricted from food storage and food preparation areas.

Method of Handling Weighbacks

Trays were checked at all meals by a dietitian and two checkers. One checker was stationed at the disposal unit to check all trays for leftover food, empty cans, cartons, and wrappers before they were placed in the respective refuse cans.

Another checker and a dietitian were stationed at the weighback table. As each test subject reported with unconsumed food, the dietitian scraped the individual items into a tared bowl; the checker recorded the net weights in the return column. Trays were then marked with a red pencil to indicate that they were ready for disposal.

Calculations

Menus. Analytical nutritive values for all ration items were obtained from the tables prepared by Dwyer and Spector.[1] Data for bread, butter, whole milk, and dry cereal were obtained from the "Table of Food Composition for the Armed Forces," U. S. Department of Agriculture.[2,3] Figures for fresh meats and potatoes were based on raw figures obtained from the above table.

Individual Dietary Data. Daily accounting of the food returned was made on the individual test subjects. The figures for the

unconsumed food were obtained for each man from the meal check sheets, and calculations were made for calories, protein, fat, carbohydrate, and ascorbic acid. The summation was then subtracted from the total day's menu to obtain the nutrients consumed by each man.

When fresh meats were served (during the rest and recovery period and the low calorie period), allowances for drippings, bone, and fat were made. The individual portion was based on an average figure. The day's menu was then adjusted for all nutrients before the individual returns were subtracted for total consumption.

Composition and Administration of Capsules

The vitamin supplement used in this experiment was based upon a formula devised by the National Research Council Committee on Therapeutic Nutrition.[4] The only alteration made in the formula as proposed by the Committee was to increase the calcium pantothenate from 40 to 80 mg. The composition of the capsule was as follows:

Thiamine HCl	10 mg.
Riboflavin	10 mg.
Niacinamide	100 mg.
Calcium Pantothenate	80 mg.
Pyridoxine HCl	40 mg.
Folic Acid	2.5 mg.
Ascorbic Acid	300 mg.
Vitamin B_{12}	4 μg.

This capsule was given four times a day to the test subjects with code numbers ending in an even digit, the supplemented group. The test subjects with code numbers ending in an odd digit, the control group, received four times a day a capsule of the same size but containing only 6 mg. of ascorbic acid and no other vitamins. The capsules given to the supplemented group were orange in color, those given to the control group were brown.

Administration of capsules was begun on January 7, 1953 and continued through the morning of March 10, 1953, or a total of 63 days.

Capsules were issued four times daily, immediately preceding each meal, including the evening snack. When capsules were missed, such as during a forced march, extra capsules were issued at the next meal.

The issuance of capsules and the supervision of their ingestion was carried out by a group of officers assigned by roster to provide one officer at each meal. Platoon leaders assisted in supervising capsule issuance and ingestion. The platoons filed into the mess hall in numerical order, all odd-numbered men first and then all even-numbered men. The platoon lieutenant was given his capsule first and he then stationed himself alongside the officer issuing the capsules. Each test subject, after providing himself with a cup of water, stepped up to the table where capsules were issued and called out his code number and name. As the officer issued the capsule he checked off the subject's name on a check sheet which provided one space for each man for each capsule administration. The platoon lieutenant ascertained that the capsule was swallowed before the man was permitted to proceed to the mess line.

Assignment of Test Subjects to Treatment Groups

To establish the separation into the two test groups the following steps were applied:

1. The platoon leaders and platoon sergeants were assigned to squads to bring each squad to a strength of 7 or 8.

2. (a) Eight-man squads were split 4 and 4 as to odd and even and such squads were given potential numbers from 1 through 8.

(b) Seven-man squads were selected to have either odd or evens predominate according to the flip of a coin. If odds predominated potential numbers were 1 through 7; if evens predominated potential numbers were 2 through 8. By chance, the difference in numbers of odds and evens for each platoon and for the entire company did not exceed one.

(c) Twelve sets of numbers from 1 through 8 were taken from the Interstate Commerce Commission's "Table of 105,000 Random Decimal Digits." Those digits not applying to given squads were scratched off, and the randomly selected numbers then applied to the roster. The digit assigned to each man became the third digit of a code number, the

first digit designating platoon number, the second digit designating squad number. There were 43 odds and 44 evens. One of the men in the odd group was ill during more than half of the experiment and all observations on him were excluded.

This technique of randomization of treatments within platoons served to insure an equal distribution of all factors, other than vitamin administration, between the two groups of test subjects. The final decision as to which group (odd or even) was to receive the supplemented capsule was made with the toss of a coin which resulted in its assignment to the even group. Within several days after assignment to test groups the test company was subjected to anthropometric measures and given a battery of tests, including the Army physical fitness test, and the Harvard step test. The results of these tests were analyzed and appear below:

	Odds (42)		Evens (44)	
	Mean	S. D.	Mean	S. D.
Age, years	24.17	4.47	24.41	4.48
Height, cm.	174.62	6.68	173.44	6.70
Weight, Kg.	70.65	10.40	72.67	9.00
Army physical fitness test (score)	177.55	58.53	164.48	62.88
Harvard step test (index)	55.17	18.71	50.23	18.96

Statistical analysis demonstrated that no significant difference in these factors existed between the two groups, indicating that the randomization process had yielded reasonably similar groups and that an aberrant distribution such as may occasionally result from random selection had not occurred.

REGULATION OF CLOTHING AND EXPOSURE TO COLD

An attempt was made to apply cold stress to the test subjects in three distinct ways. Uniforms which were lighter than required for complete comfort were prescribed for all daytime activities. During a period of 5 weeks the barracks fires were banked through the night so that they provided less heat during the hours of sleep than during the day. Cold exposure tests were conducted on four occasions; men at rest were subjected for periods of several hours to cold environments, with adequate protection of sensitive areas (face, hands, feet).

Prescribed Uniforms

In order to produce roughly standardized exposure to cold, the uniform for wear in the activities between 0700 and 1700 hours was prescribed daily beginning January 25, 1953. The type designated for a given day was based upon a prediction of temperature made the previous evening by the weather observer. Modification was made in the morning or at noon if necessary according to actual weather conditions.

Four classes of uniforms were designated A, B, C, and D. The maximum clothing permitted with each of them was prescribed. Less clothing was permitted *ad libitum*. As prescribed, the uniforms were sufficiently light to cause a man to become cold after a few minutes of inactivity outdoors. Since the test subjects were active most of the time when outdoors, they were usually not uncomfortably cold under this arrangement. On February 27, in conjunction with institution of other measures to increase stress, the next lighter uniform was prescribed for each temperature range. This system of prescribing uniforms resulted in men becoming cold almost instantly when inactive and at least cool during light activity. However, it was found that it was not possible to make a very active man cold by restriction of clothing in the weather conditions encountered in this test. Because of the generally high activity of the test subjects it is doubtful that any considerable degree of stress from cold was achieved by regulating the uniforms.

Control of Barracks Temperatures

From January 20, to February 23, fires in the barracks were banked at night so as to give off little heat, but to preserve a bed of coals for morning. Fires were stoked again after 0500. Continuously recording thermographs were placed in the center of each barracks, between the two stoves. These were in the warmest regions of the barracks. Men

sleeping at the periphery of the building, particularly on the windward side, were subjected to moderately lower temperatures. Although the minimum temperature, which occurred between 0500 and 0600 hours, was occasionally near 30° F., the mean of the minimum temperatures was in the mid-40's. Mean temperatures were calculated from the continuous records for the period between 2200 and 0600 hours. In general, the weekly mean night temperatures were near 60° F.

Each man was provided with a single mountain-type sleeping bag. The clothing the men slept in was not regulated, but most of the subjects slept in long woolen or cotton underwear.

Cold Exposure Tests

These tests are described in a later section. They consisted of periods of 2 to 4 hours of exposure to a cold environment indoors or outdoors. Test subjects became uncomfortably cold during these tests, as manifested by complaints, shivering, and decreases in rectal temperature. Each man was subjected to this exposure on four occasions.

PROGRAM FOLLOWED BY TEST SUBJECTS

The test subjects followed a prescribed program of activity each day. The types of scheduled activity can be conveniently divided into the following: activity procedures, test procedures, and guard and work details.

Activity Procedures

Except where testing procedures interfered, it was attempted to have about seven hours per day of moderate to high activity, primarily consisting of marching, sports or drill, or calisthenics. In general, only a limited number of sports activities were possible. These were as follows: touch football, basketball, capture the flag, and skiing.

The weekly scheduled programs were analyzed and classified into nine types of days. During all the days comprising one type of day the distribution of scheduled hours for various kinds of activities was approximately the same. In Table IV are presented the average number of hours scheduled in the various activities on various types of days. At the bottom of the table appear the number of days that each type of day occurred in the schedule. In the preparation of this table three of the weeks were excluded. The first week was excluded since the activity of the men was not scheduled but rather a progressively increasing activity program was carried out by the platoon leaders. The sixth and tenth weeks are excluded because they were weeks of rest and rehabilitation.

Since the maintenance of the camp necessitated the use of test subjects on work details, the type of activity from which men could be drawn for this purpose was prescribed. Otherwise, the men were excused from the prescribed

TABLE IV

Activity	Type of day, hr.								
	1	2	3	4	5	6	7	8	9
March	3	4	4	—	—	—	—	3	4
Sports or drill	3	3	4	3	4	3	—	—	—
Calisthenics	1	1	—	1	—	—	—	—	—
Low activity	—	—	—	4	4	4	0–4	—	—
Commander's time	1	—	—	—	—	—	—	5	4
Forced march	—	—	—	—	—	—	4–10	—	—
Physical fitness tests	—	—	—	1	1	1	—	—	—
Physical examination	—	—	—	—	—	1	—	—	—
Sleep	8.5	8.5	8.5	8	8	8	8.5	8.5	8.5
Eating	1	1	1	1	1	1	0.5–1	1	1
Toilet	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Off duty, meal hours	2	2	2	1.5	1.5	1.5	1–1.5	2	2
Off duty, evenings	4	4	4	4	4	4	4	4	4
Daily total	24	24	24	24	24	24	24	24	24
No. of days of this type	12	6	3	6	6	2	7	4	3

SCHEDULE OF WEEKLY TESTS

	PRE-TEST	STRESS 4000 CALORIES					REST	STRESS 2500 CAL.		STRESS 2000 CAL.	REST
WEEK	0	1	2	3	4	5	6	7	8	9	10
MEDICAL EXAMINATION											
ANTHROPOMETRIC MEASUREMENTS											
PSYCHOLOGICAL TESTING											
WEIGHT											
COLD TEST, INDOOR											
COLD TEST, OUTDOOR											
PHYSICAL PERFORMANCE											
FORCED MARCH											
CONTEST MARCH											
ARMY P T TEST											
HARVARD STEP TEST											
HAND DYNAMOMETER											
BIOCHEMISTRY											
HEMOGLOBIN											
EOSINOPHILS BASAL LEVEL											
BLOOD ASCORBIC ACID											
BLOOD SUGAR											
URINE ASCORBIC ACID 24 HOUR TOTAL											
URINE NITROGEN											
URINE 17 KETOSTEROIDS 24 HOUR TOTAL											
URINE URIC ACID/CREATININE											

Fig. 1. Schedule of weekly tests.

activity only because of reporting to sick call, confinement to quarters, or hospitalization. A concerted effort was made by the medical officers to arrange compensating activity for the time missed by the men reporting to sick call who were deemed capable of performing the prescribed physical activity. Rather than lose the man from the study or unnecessarily prolong his absence from the planned activities, some of the men were given short-term periods of low activity because of blisters, internal derangements of the knee or ankle, etc.

Recreational activities which further increased the energy expenditure of the subjects, primarily skiing to tobagganing, were encouraged.

Test Procedures

In Figure 1 are indicated the weeks in which the specific test procedures were done for the measurement of physical performance, biochemical changes, and the miscellaneous tests. During the first week all of the test subjects were given a complete physical examination. This was repeated during the second week, with emphasis upon the nutritional status of the individuals. Repeat physical examinations were given at the fifth week, at the end of the 4000 calorie intake phase, and again at the tenth week following the period of restricted caloric intake. Anthropometric measurements of the subjects were done at the time of each physical examination. Psychological testing was done at the end of the second week, just prior to the second phase; at the end of the fifth week, at the end of the second phase; and at the tenth week, at the end of the fourth phase of the study. Body weight was taken at least once weekly during the entire study. The indoor cold test was given only once, during the seventh week. Outdoor cold tests were given once each week during the seventh, eighth, and ninth weeks.

The tests of physical performance used in this study, the Army physical fitness test, the

Harvard step test, and the hand dynamometer, were performed by the subjects just prior to the start of the study, and weekly thereafter. In the scheduling of the Harvard step tests and Army physical fitness tests, it was attempted to have all of the men under the same conditions at the time of the test. To this end, one hour was allowed after breakfast before the testing began, and the platoons which were not scheduled to take the test until later were kept at low activity in the barracks. The order in which the platoons took these tests was rotated each week. Also, since it was desired to have all of the men undergoing the same energy expenditure daily, the men who had already taken the test were kept at low activity for the same length of time as the men who took the test last.

The forced march, another test of physical performance, was done during weeks 2 through 5, inclusive, and weeks 7 through 9, inclusive. A contest march was performed during the eighth week.

The biochemical determinations can be divided into two categories: first, those employed primarily to measure the nutritional status of the test subjects and, second, those determinations employed primarily to attempt to measure the degree of stress to which the subjects were exposed. Under the first category, the 24-hour urine ascorbic acid excretion, the blood ascorbic acid, and the hemoglobin were measured prior to the start of the study, and weekly during weeks 1 through 9, inclusive. The blood sugar was measured prior to the start of the study, and during weeks 1 through 6, inclusive. The 24-hour nitrogen excretion in the urine was measured prior to the start of the test, and during weeks 3 through 9, inclusive. Under the second category, the basal level of eosinophils and 24-hour urine 17-ketosteroid excretion were measured prior to the start of the study and weekly during weeks 1 through 9, inclusive.

In addition, selected determinations were done in conjunction with specific stress procedures to which the test subjects were exposed. On these days the pre- and post-stress levels of eosinophils were obtained, and three fractions of the 24-hour urine excretion (overnight, stress, and post-stress) were collected for 17-ketosteroid excretion, uric acid/creatinine ratio, and ascorbic acid partition (oxidized and total).

Each of the test procedures mentioned above is further elucidated in the section on Methods and Results.

Guard and Work Details

It was necessary to use the test subjects for work details and for guard duty in order to maintain the campsite. One platoon per day was assigned to guard and work detail. The assignments were rotated so that each platoon had the same number of total days of assignment during the course of the test. On the days that their platoon was assigned to these details, the members of the platoon were excused from the performance of the regularly scheduled physical activity; however, they were not excused from the scheduled test procedures.

Platoons were assigned to guard and work detail for the period from 1800 to 1800 daily. During that time they were responsible for the guard detail from 1800 to 0600, then were responsible for the provision of men for the dining room and work details from 0600 to 1800 of the following day. During the period of guard duty, the men were on duty at their posts for a period of two hours; they were off duty for four hours, then had a second period of duty for two hours. During the period on duty the men were walking an assigned post, or were tending fires in the buildings assigned to their post.

The daily work details were performed by those men not assigned to dining room detail. The provision of water for all buildings was one of the main duties. This entailed carrying 5-gallon cans until Lister bags were filled, supervising the filling of a water trailer, etc. Other details were responsible for the loading and unloading of coal for distribution to all the buildings, the maintenance of fires, the provision of fire guards, disposal of ashes, garbage, etc. The duties of the dining room detail consisted mainly of washing trays and silverware, cleaning dining tables, and unloading mess supplies.

In addition to the ordinary work details and dining room details, a different platoon than the one assigned to these duties was assigned to a special work detail. These special work details were instituted on January 19, and continued through March 6. These details were also rotated exactly through the four platoons. The special work details were for one or two hours daily and replaced sports and drill on the daily schedule. During this time the men were assigned a specific task, such as digging the sanitary fill, repairing or refurbishing buildings, snow removal on the roads leading to the camp site, etc.

Methods and Results

In the analysis of results attention has been concentrated on assessing differences between the two treatment groups. Other features of the data that do not pertain to differences between the treatment groups have received only incidental consideration. Several of these points are of interest and further analyses of the data in regard to them will be dealt with in future reports.

Interrupted Participation in the Experiment by Certain Test Subjects and the Acceptability of Data on These Subjects

As had been anticipated, many of the test subjects were more or less incapacitated for short intervals because of illnesses such as respiratory infections, minor injuries, and gastrointestinal upsets. Analysis of the morbidity records is presented in another section as a type of comparison between the control and supplemented groups. During these illnesses there was often interference with the taking of physical performance and metabolic data and the maintenance of the prescribed high rate of caloric expenditure and cold exposure. At times the subject was left in a weakened condition so that immediately succeeding tests were not well performed. It is neither necessary nor desirable to eliminate data from all subjects temporarily incapacitated by illness or injury. The time of illness was generally insignificant compared to the total duration of the test. Subsequent performances demonstrated that the period of recuperation was also short. The percentage of subjects on whom some observations were missed in the course of the experiment was great enough so that their elimination would drastically interfere with the statistical analysis of data. In a sense, the illnesses and injuries in themselves may be considered as additional physical stresses not foreign to the soldier in combat.

In several instances, prolonged or unusual interruptions of participation in the experiment required special consideration. The medical officers present during the experiment reviewed all of these cases afterwards from the standpoint of acceptability of data. One man was dropped from the study entirely, and specific tests were excluded from consideration in the case of two other men. These decisions were made on the basis of medical and performance criteria, and the effect they might have had on any subsequent analysis did not enter into the decision.

With the exceptions noted above, all other scores on performance tests were included in the analysis of the data. Some men were excused from one or more of the tests because of illness, but, with the exceptions just cited, the decision to exclude the data was never made after a test.

Measurement of Factors Influencing Results

Food Intake

A record of the food intake of each subject for each day of the experiment was kept as described under the section on Dietary Regimes. From these, weekly averages for each man for caloric, protein, and ascorbic acid intake were computed. From these weekly individual-man averages, the mean and standard deviations of the intake for the control and supplemented groups were calculated. These data on intake, together with the amount offered in the diet, are presented in Tables V, VI, and VII. The first week represents a 5-day period because the diet was not started until the third day of that week. The eighth week is divided into two periods because the diet was changed from 2500 calories offered to 2000 calories offered on the fifth day of this

TABLE V
Mean Caloric Intake (Calories per Day)

Week	No. of days	Amount offered in diet	Intake			
			Control		Supplemented	
			Mean	S. D.	Mean	S. D.
1	5	4138	3603.8	463.76	3620.3	372.12
2	7	4138	3554.1	480.14	3379.1	565.47
3	7	4138	3565.6	448.50	3429.0	461.53
4	7	4138	3479.7	438.11	3351.3	559.19
5	7	4138	3507.4	455.69	3387.5	467.28
6	7	4047	3607.0	381.83	3609.6	312.07
7	7	2551	2499.9	74.45	2488.1	61.87
8	4	2600	2562.6	34.09	2554.0	56.18
8	3	2032	1979.5	53.37	1989.0	46.43
9	8	2032	1979.5	53.37	1989.0	46.43

TABLE VI
Mean Protein Intake (Gm. per Day)

Week	No. of days	Amount offered in diet	Intake			
			Control		Supplemented	
			Mean	S. D.	Mean	S. D.
1	5	151	131.4	20.17	128.2	20.30
2	7	151	125.7	24.37	117.2	27.81
3	7	151	117.1	22.70	110.3	21.83
4	7	151	112.3	23.28	107.2	23.19
5	7	151	115.1	23.95	109.0	20.13
6	7	148	129.5	13.73	130.0	11.21
7	7	76	75.6	1.81	75.2	1.12
8	4	80	79.6	0.35	79.2	1.74
8	3	70	67.7	2.56	68.3	1.96
9	8	76	75.6	1.65	75.3	1.95

week. In all other weeks the diet offered was uniform throughout the week. The ninth week includes the Monday of the tenth week. All values in these tables are for 42 men in the control group and 44 men in the supplemented group.

Mean caloric intake for each week of the experiment for the two treatment groups is presented in Table V. During the first six weeks, while 4000 calories were being offered, the food intake varied considerably from man to man so that the coefficient of variation of the intakes during this period was 12.52 per cent for the control and 13.27 per cent for the supplemented group. During the last three weeks when 2500 or 2000 calories were furnished by the diet, the variability in intake decreased sharply, since most of the men were eating the entire diet. The coefficients of variation for the last 3 weeks were 1.99 per cent for the control and 2.17 per cent for the supplemented group. Throughout the course of the experiment the mean intake of the two groups differed only slightly. For the entire 9-week period the mean daily caloric intake was 3116.2 for the control group and 3051.3 for the supplemented group. The difference, 64.9 calories per day, multiplied by 62, the number of days the diet was fed, gives 4023.8 calories, the mean amount the control group ate in excess of the supplemented group for the entire experimental period. During the first five weeks, when the greatest variations in food intake occurred, the mean intake of the control group was 3542.1 calories per day and that of the supplemented group was 3433.4 calories per day. The difference, 108.7 calories per day, is not statistically significant ($t = 1.06$, $P < 0.05$).

The values for mean protein intake by weeks are given in Table VI. The intake fell progressively during the first five weeks in both

TABLE VII
Mean Ascorbic Acid Intake (Mg. per Day)

Week	No. of days	Amount offered in diet	Intake			
			Control		Supplemented	
			Mean	S. D.	Mean	S. D.
1	5	40	36.7	6.40	37.1	5.16
2	7	40	35.9	4.08	34.2	5.14
3	7	40	35.7	3.56	34.0	5.08
4	7	40	33.1	4.38	31.3	5.82
5	7	40	34.9	5.00	33.6	4.95
6	7	38	32.0	4.72	31.5	4.43
7	7	43	41.0	2.17	40.5	2.09
8	4	45	44.2	1.71	43.8	1.92
8	3	37	35.4	1.53	35.6	1.17
9	8	39	39.0	1.70	38.7	2.34

groups. This is attributable to the increasing rejection of canned meat items. Protein intake rose sharply when fresh meat was substituted (week 6), and during the period of caloric restriction (weeks 7, 8, and 9) the protein intake was almost equal to the amount offered in the diet.

Table VII presents the information on ascorbic acid intake. The values vary between 30 and 40 mg. per day for the entire experimental period. There is no indication of a difference between the two treatment groups.

Meteorological Data

Continuous records of temperature, wind velocity, and wind direction were made at the camp from January 5, to March 10, 1953. Continuous daytime records were made of duration of sunshine and intensity of solar radiation. Windchill, the resultant of temperature and wind velocity, was calculated from the equation of Siple[5] for each hour. Hourly observations of relative humidity, barometric pressure and cloud cover were made between the hours of 0730 and 2030. The weather conditions for the period of the test are summarized in Table VIII and Figure 2.

TABLE VIII
Synopsis of Weather During Period of Test

Month	Avg. daytime temp. and range of avgs.	Avg. daytime wind vel. and range of avgs.	Avg. daytime windchill and range of avgs.
	°F.	*mph.*	*Kg. cal./sq.m./hr.*
Jan. (5–31)	30 (9–44)	15 (6–21)	1000 (780–1330)
Feb.	22 (0–40)	12 (5–18)	1070 (760–1460)
Mar. (1–10)	27 (9–41)	12 (9–17)	990 (730–1220)

Activity Records

As a means of determining how closely the activities of the test subjects conformed to the scheduled program, daily records were made of time spent in various categories of activities by each platoon.

TABLE IX
Comparison of Planned and Actual Time Consumption by Activity During a Composite Day in the Fourth Platoon

Activity	Planned distribution		Actual distribution, avg.: weeks 2–5 and 7–9	
	Hr.		*Hr.*	
Sleep	8.3		8.0	
Toilet	0.5		1.47	
Eating, incl. meal hr.	2.84–2.98		3.3	
Off duty	6.04–6.61		5.61	
		17.68–18.39		18.36
Step test	0.29		0.13	
Fitness test	—		0.19	
Physical exam.	0.04		0.03	
Cold test	—		0.3	
Calisthenics	0.49		0.23	
March, daily	1.96		0.90	
March, forced	0.57–1.43		1.22	
Drill and sport	2.31		0.56	
Work	—		1.88	
		6.52–5.66		5.44
		24.20–24.05		23.80

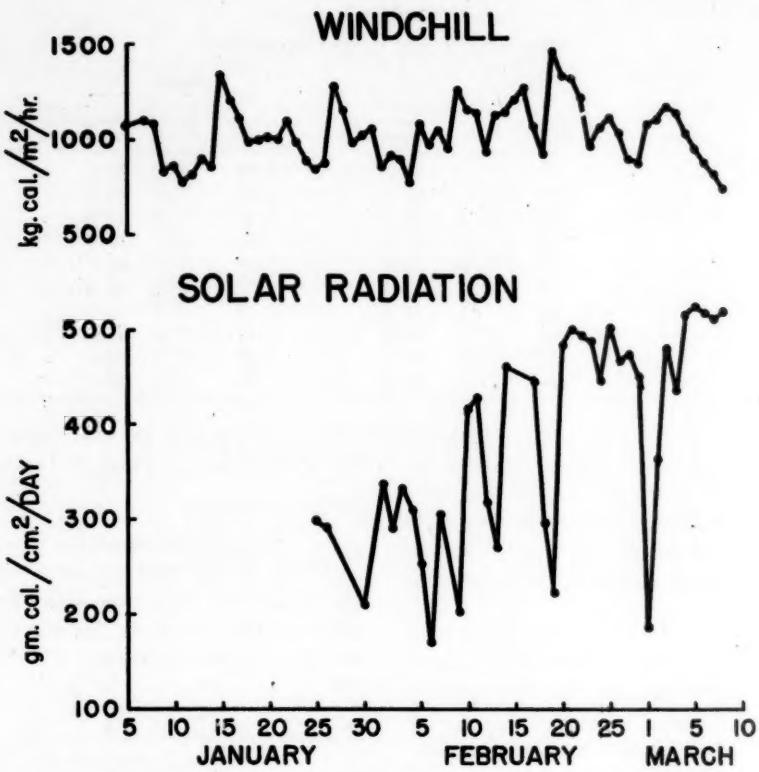

Fig. 2. Daily mean windchill and mean solar radiation during the period of the experiment.

Observations of the time-activity distribution were made daily in each of the platoons by the same observers, sergeants participating as test subjects, who recorded the observations on a form sheet delineating activities of various categories with a continuous line. Table IX compares the observed times of activities with the planned times according to the type of activity for platoon IV. Comparable data were obtained on the other platoons. The average daily time-distribution of activities given in this table was calculated by totaling the daily time-activity records for each period and then deriving a mean value. The resultant composite day is fictitious inasmuch as all the activities were never scheduled for the same day. As planned, about 75 per cent of the total 24 hours was occupied with low energy activities which were concerned primarily with the maintenance of the individual, while the remainder of the time, 25 per cent of the day, was devoted to moderate to high energy activities. It is seen that the percentage of time devoted to the higher energy cost activities approximates the planned value. Work details necessary for the function of the test camp occupied about one-third of the time devoted to moderate and high energy activities.

Energy Balance

The energy expenditure under the conditions of this experiment can be assessed from the data on caloric intake and body weight. If body weight remains constant, caloric intake may be considered equivalent to energy expenditure, providing no important changes in water balance are occurring. There is no reason to suspect that changes in water balance occurred during the present study and during the first five weeks the mean body weight of the entire test company fell 0.9 Kg. The mean

caloric intake during this period was 3500 calories per day, so the caloric expenditure may be assumed to be at least equal to this value. The caloric equivalent of weight loss varies with a number of factors; however, under the conditions with which we are dealing, 6600 calories per Kg. is a reasonable working estimate.[6] Thus, the 0.9 Kg. weight loss may be given a caloric equivalent of about 6000 calories for the 5-week period, or about 170 calories per day. Therefore, the total mean caloric expenditure may be placed at about 3700 calories per day. When account is taken of the fact that the activity schedule was based on a week with 6 days of high activity whereas this calculated value is an average for a 7-day week, it will be seen that energy expenditure closely approximated the planned level. During the period of caloric restriction the mean intake for the 22 days was 2300 calories per day and the mean weight loss during this period was 3.9 Kg. with a caloric equivalent of 1170 calories per day, which, when added to the caloric intake, gives a calculated mean daily expenditure of about 3500 calories.

MEASUREMENT OF PHYSICAL PERFORMANCE

During the present study a high level of motivation was fostered through the recognition of individual excellence and the stimulation of inter-platoon competition. Platoon scores and lists of individuals attaining high scores were posted within a few hours after each test. Each man could review his past performances frequently and strive for improvement. "Cheer leaders" spontaneously developed within the platoons and goaded their reluctant team-mates into "giving their all." It was the opinion of several experienced observers that motivation was much better in the present study than they had observed in prior tests under field and laboratory conditions.

Harvard Step Test

The Harvard step test[7,8] was administered to all subjects at approximately weekly intervals for a total of 11 tests, except when a man was unable to perform due to illness or injury.

Procedure. According to the original description,[7] the step test ". . . consists in having the subject step up and down a 20 inch platform 30 times a minute for five minutes unless he stops from exhaustion before then. . . . Each subject stands at attention in front of the platform. Each observer stands behind his subject. At the signal *up*, the subject places one foot on the platform, steps up placing both feet fully on the platform, straightens his legs and back and immediately steps down again one foot at a time." In all the present tests the cadence was measured with a metronome and counted, "Up-Down-Up-Down," the "Up" coming every two seconds. The description continues ". . . it is easier for the subject to 'lead off' with the same foot each time and not try to alternate the feet, but this can be done two or three times during the test if one leg gets tired. . . . The observer must be sure that the subject steps fully on the platform and takes a standing position at each step up. No crouching should be allowed. The subject must keep the pace accurately and if he falls behind because he is tired the observer must stop him after he has been unable to keep the pace for 10 or 15 seconds." During the present study exceptions were occasionally made when the posture or cadence did not strictly comply with these rules, due to body build or natural clumsiness. Subjects were allowed to stop for one to three cycles to regain lost rhythm provided they did not continue to repeat this because of exhaustion. It was felt that the maintenance of a high level of energy output for as long a period as possible was of more importance than slavish insistence on perfect form. The form of individual men was generally consistent from week to week.

Subjects performed these tests wearing long woolen underwear, socks, and rubber sneakers. One squad was tested at a time. About three hours were generally required to test the entire company.

The time in seconds was recorded when the subject stopped from exhaustion or at the end of five minutes (300 seconds). The subject then sat quietly on the bench. A physician or trained technician counted radial or carotid pulses during the intervals 1 to $1^1/_2$ minutes,

2 to $2^1/_2$ minutes, and 4 to $4^1/_2$ minutes after exercise.* The pulses counted in these three intervals were summed and multiplied by two to give the sum of three 1-minute pulse rates.

Scoring. The following formula for scoring was presented by the originators of this test:[8]

$$\text{"Index of Fitness"} = \frac{\text{Duration of exercise in seconds} \times 100}{\text{Sum of three post-exercise pulse rates}}$$

The calculated index is heavily weighted by the duration of exercise if less than five minutes. This was recognized by the originators who stated, "Duration of the effort before exhaustion is given a prominent place in our index for two main reasons. First, endurance or 'staying power' in a man has always been highly esteemed, and is an indication not only of stamina but also of strength, skill, and determination. Second . . . duration and intensity of effort and one other measurement allow one to approximate the . . . (other physiologic indices of fitness). . . . In the present test, intensity is fixed, and the recovery pulse rates are counted. Hence, duration must be measured." [8] The weight given to duration of exercise in the formula is arbitrary. Exercising for only two and a half minutes will not give a post-exercise pulse rate half of that obtained following five minutes of exercise as the formula would suggest.

Duration of exercise cannot be used as an independent measure of fitness because exercise was arbitrarily halted at five minutes. There is no way of knowing how much longer than five minutes men completing this duration could have performed before reaching exhaustion. The percentage of test subjects completing five minutes is indicated for successive tests in Table X and Figure 3, parts 1 and 2. It is noted that only 26 per cent of the vitamin supplemented and 39 per cent of the control groups completed five minutes during the first test, whereas more than 80 per cent of each group finished most subsequent tests and 98 per cent completed the final test. It is impossible to ascertain relative improvement in stamina to high energy output work with this test, as practically all men were halted before reaching exhaustion in the final tests. The fact that in the initial test the percentage of men completing five minutes was smaller in the supplemented than in the control group gives the appearance of greater improvement in the supplemented group. Because the "index of fitness" formula gives strong weight to the duration of exercise, the appearance of greater improvement in the supplemented group is also evident in the "index of fitness" scores. Further analyses using this index do not appear warranted.

Post-exercise pulses alone are likewise unsuitable as measurements of fitness unless they follow exercise of uniform intensity and duration. Where the duration is less than standard, it should be possible to correct pulse rates to a value approximating that which would occur had the subject completed the full five minutes. Appropriate methods of correction are not found in the literature. The data collected in the present study permitted the construction of a suitable correction formula.

In many instances a man would complete the full five minutes on one test but would not exercise for this long a period on an immediately preceding or following test. Allowing for the mean training effect observed in sequential tests, it was possible to estimate for shorter periods of exercise what the pulse rates would have been had the exercise lasted five minutes. An example of the mathematical process involved is given below:

Preliminary Data for All Men

A. Test number	1	2	3	4
B. Mean pulse sum of all men completing five minutes	405	400	390	385

* The use of these particular pulse intervals was recommended by Johnson, *et al.*[8] as being representative of the entire pulse decay curve. In applying the "index of fitness" formula to the step test, Brouha substituted the 3 to $3^1/_2$ minute pulse for the 4 and $4^1/_2$ minute one.[7,9] This substitution changes absolute values slightly but should have no significant effect on relative scores within a series. Other members of the original "index of fitness" team have retained the 4 to $4^1/_2$ minute pulse. We have followed their example.

TABLE X

Summary of Harvard Step Test Data

Phase of study →			Control	Equilibration			High intake, high output			Rest	Low intake, high output		
Test number* →			1	2	3	4	5	6	7	8	9	10	11
Days after capsules started →			−1	+3	+10	+17	+22	+27	+34	+41	+48	+55	+62
Number of men tested	Control		41	40	41	40	41	38	40	40	39	39	40
	Suppl.		43	43	42	42	41	43	43	44	44	44	43
Pre-exercise pulse rate†	Control	Mean	93.9	91.5	91.3	87.0	90.1	85.3	86.0	83.2	79.1	75.3	74.1
		S. D.	10.5	10.5	10.2	10.1	10.4	10.7	12.1	11.4	13.3	12.8	13.9
	Suppl.	Mean	97.4	97.7	92.3	91.5	92.4	85.0	85.5	82.0	81.5	74.6	73.1
		S. D.	8.9	9.7	11.6	11.1	9.4	13.5	10.3	10.2	10.8	10.1	10.5
Men completing five minutes	Control	Per cent	39	90	66	78	85	82	90	88	90	95	98
	Suppl.	Per cent	26	72	52	79	90	88	93	95	98	98	98
"Index of fitness" score	Control	Mean	54.2	71.2	65.7	72.7	73.7	78.1	78.5	78.5	83.6	90.0	90.9
		S. D.	18.5	11.6	23.6	15.9	16.3	18.6	17.5	16.4	18.1	20.5	20.0
	Suppl.	Mean	49.1	65.7	59.9	70.3	74.6	77.4	79.7	81.8	86.0	92.6	94.2
		S. D.	18.5	14.1	18.9	13.1	9.1	12.2	12.9	8.9	10.5	16.4	16.4
Corrected sum of post-exercise pulses	Control	Mean	415.8	406.3	397.9	377.3	382.6	361.7	364.9	363.6	346.8	325.5	323.4
		S. D.	32.9	33.1	36.8	28.4	31.8	33.1	36.4	31.8	36.7	40.0	38.0
	Suppl.	Mean	421.8	415.6	398.5	396.4	388.5	372.6	369.9	363.5	348.6	324.6	316.8
		S. D.	40.3	35.1	32.2	31.7	21.2	29.0	38.5	31.1	36.8	41.5	41.4

* Tests done early in a particular week are considered representative of the previous week's condition.

† Where individual tests were missed, values midway between preceding and succeeding determinations were interpolated. At beginning and end, values equal to nearest determination were utilized. Weekly values hence are for 42 control and 44 vitamin supplemented subjects.

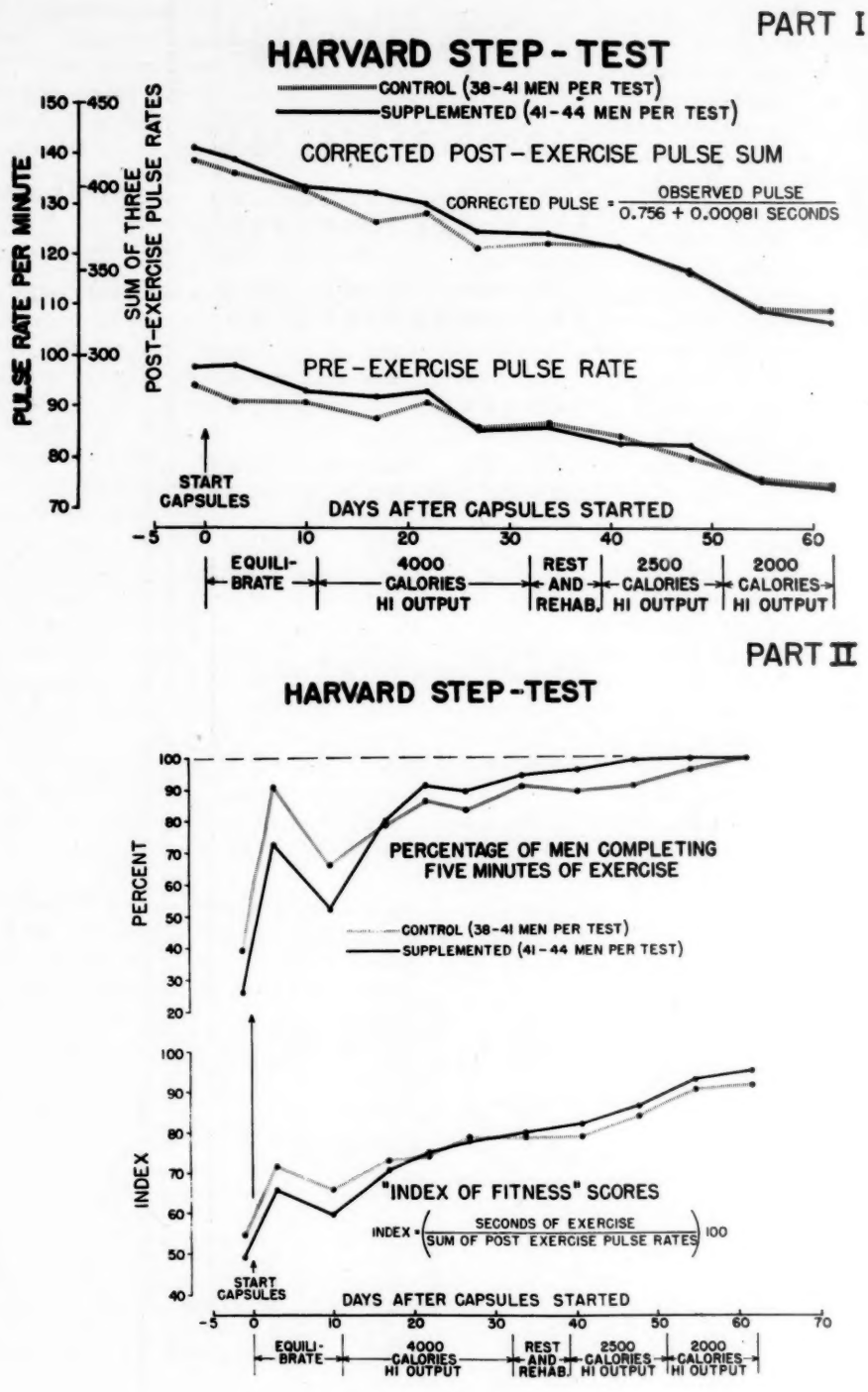

Fig. 3. Harvard step test data.

C. Pulse as percentage of prior test pulse (e.g., $100 \times B_2/B_1$)

...	99	98	99

D. Pulse as percentage of succeeding test pulse (e.g., $100 \times B_2/B_3$)

101	103	101	100

Representative Data from Particular Man (#121)

E. Observed duration of exercise in seconds

88	300	132	300

F. Observed pulse sum

344	424	340	390

G. Predicted pulse from prior test (e.g., $F_2 \times C_3$)

...	...	416	...

H. Predicted pulse from succeeding test (e.g., $F_2 \times D_1$ and $F_4 \times D_3$)

428	...	394	...

I. Mean predicted pulse sum (e.g., $G_3 + H_3)/2$

428	...	405	...

J. Observed pulse as percentage of predicted (e.g., $100 \times F_3/I_3$)

80.4	...	84.0	...

The observed pulse as a percentage of the predicted pulse (J) was then plotted against the observed duration of exercise (E) for the first and third tests in this case. Ninety-nine such instances were present in the data of this study, and each such instance was plotted as is shown in Figure 4. Most such instances occurred in the early weeks of the study; the distribution of values did not appear to vary significantly whether they occurred early or later in the course of the experiment. A regression line passing through the value of 100 per cent at 300 seconds was derived statistically:*

$$\text{Percentage of five minute pulse sum} = 75.6 + 0.081 \times \text{time in seconds}$$

This formula was further refined so that the corrected pulse sum can be estimated from the observed pulses and the duration of exercise.

$$\text{Corrected pulse sum} = \frac{\text{Observed pulse sum}}{0.756 - 0.00081 \times \text{seconds}}$$

Table X and Figure 3 give the mean corrected pulse sums for all step tests of the vitamin and control groups.

Other methods of scoring have been suggested. Rate of post-exercise pulse decay and ratio or difference of pre- and post-exercise pulse rates have been thought significant.[8,10] In the pre-supplementation test of this series the contours of post-exercise pulse decay curves were not related to other indices of fitness. Neither was any striking relationship evident between pre-and post-exercise pulse rates.

Over the course of the entire experiment, however, the mean pre-exercise pulse rates progressively decreased at about the same rate as did the average of three post-exercise pulse rates (see Figure 3 and Table X). These were not truly basal pulse rates, as the subjects generally would be sitting for only two minutes or less before they were taken. Frequently the subjects would be tested shortly after walking from their barracks and disrobing. No further analyses of pre-exercise pulse rates were felt warranted.

In Figure 5 is presented the mean pulse response in 3 step tests (second, seventh, and eleventh weeks) of 55 men (25 supplemented, 30 control) who completed 5 minutes of exercise in each of these tests. This graph demonstrates that although the resting pulse rates and post-exercise pulse rates show a progressive decrease with time, the slope of the post-exercise pulse rate decay curve is not altered, i.e., the post-exercise pulse rate curves are essentially parallel. Furthermore, no striking difference between the slopes for the control and supplemented groups is evident.

Analysis of Results

The trends of each group with regard to percentage completing 5 minutes, "index of fitness" scores, and corrected post-exercise pulse sums were discussed in the previous section. Of these, the corrected post-exercise pulse sums are the only suitable measure for comparison of changes in the two groups occurring over the time of the experiment.

The most efficient generally accepted method for the statistical evaluation of such data is through an

---

* $\Sigma(t - X)^2 = nt^2 - 2t\Sigma X + \Sigma X^2$.
$\Sigma(t - X)(p - Y) = ntp - t\Sigma Y - p\Sigma X + \Sigma XY$.
$b = \dfrac{\Sigma(t - X)(p - Y)}{\Sigma(t - X)^2}$.

where (t, p) is the fixed pivot point ($t = 300$, $p = 100$), X represents individual time observations, Y represents individual percentage observations, b is the slope of regression, and n is the number of observations.

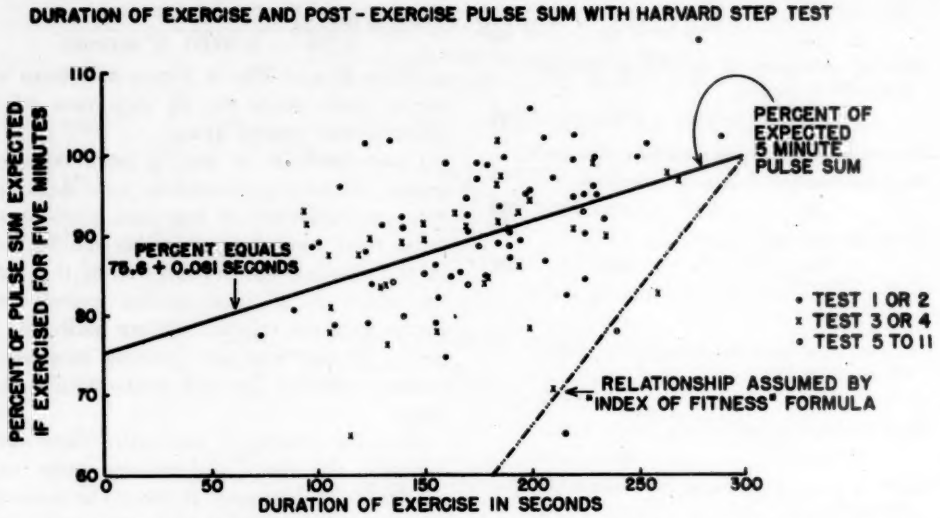

Fig. 4. Regression of observed pulse sum as percentage of 5-minute pulse sum on duration of exercise in Harvard step tests in which duration of stepping was less than 5 minutes.

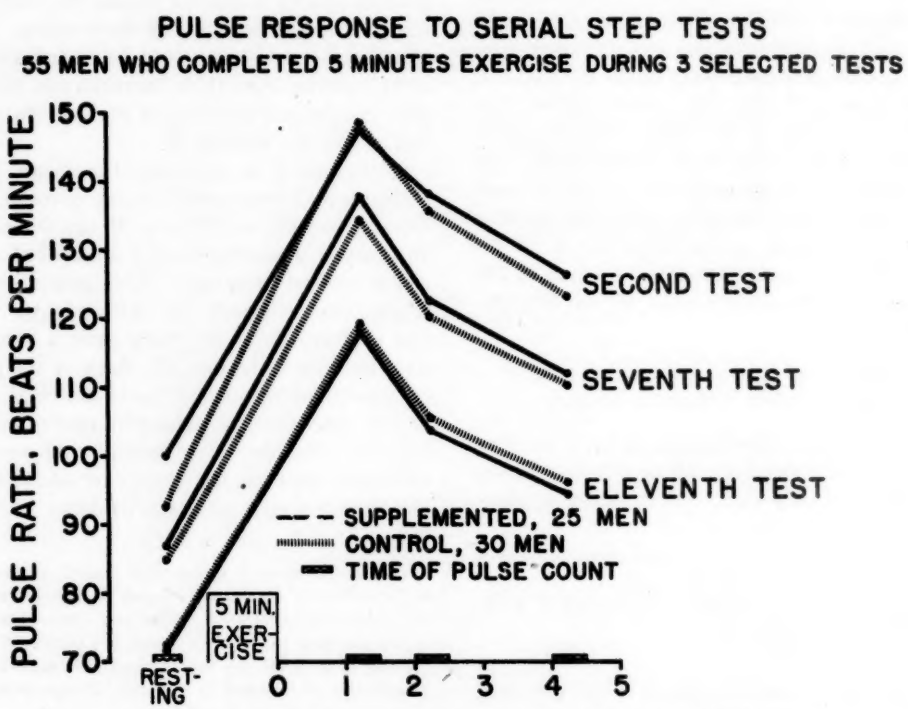

Fig. 5. Mean pulse response in selected serial Harvard step tests.

analysis of covariance using the final test as the variate and the pre-treatment test as the covariate. This analysis minimizes initial difference in fitness between men (age, training, obesity, body build) by adjusting the final score according to the first. Analysis of the arithmetic difference between first and last tests is but a special case of this technique and need not additionally be performed. Differences between platoons due to leadership and other considerations are also minimized by this technique.

An analysis of covariance was carried out using the corrected pulse sum of the last test as the variate and that of the first test as the covariate (Table XI, Analysis 1). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was 9.26 with the control group having the higher value, and 95 per cent confidence limits of +25.45 and −6.93. Translated into post-exercise heart rate, the supplemented group had an adjusted mean of 3 beats per minute less than the control group. The F ratio of this difference was 1.295, giving a P value of 0.23 which is not statistically significant.

Another analysis of covariance was carried out using the corrected pulse sum of the seventh test (end of high calorie, high activity period) as the variate and the first test as the covariate (Table XI, Analysis 2). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was −2.24 with the supplemented group having the higher value, i.e., higher post-exercise pulse rate. The 95 per cent confidence limits of this difference are +12.96 and −17.44. The F ratio for this difference was 0.086, giving a P value of 0.77, which is not statistically significant.

Although the first and last tests only are generally used in such an analysis, some method of incorporating data from intermediate tests would seem more efficient. It is evident from Figure 3 that the regression of mean corrected pulse rates is nearly linear with respect to time.

Individual men, however, show fluctuations in corrected post-exercise pulse sums unrelated to the long time trends. It appears that the intersect of a statistically derived regression line with any particular week of the study will give a more precise estimate of the mean value expected, were many tests run in that week, than does the single test actually performed. From such a regression line one may estimate with greater precision the expected values for the first and eleventh tests for use in the analysis of covariance. Two theoretical objections may be raised to this procedure: (1) The position of the pre-supplementation intersect is determined in large part by post-supplementation test results; it is not, therefore, a true control. However, if there is a constant rate of change from the control, extrapolation back from post-control values should intersect the true control level. (2) Difference in the two groups occurring late in the course of the experiment might be masked by the influence of earlier observations. These two possibilities can be crudely tested in this instance by inspection of the time trends of Figure 3. The initial and final mean values do not deviate appreciably from the linear relationship of the intermediate points. It was therefore felt warranted to calculate a regression of each subject's results against time.

An analysis of covariance was carried out using the intersect of the calculated regression with the tenth

TABLE XI
Summary of Analyses of Covariance

Analysis no.	Name of test	Variate	Covariate	Unadjusted means					
				Control			Supplemented		
				N	Variate	Covariate	N	Variate	Covariate
1	Step test	10th wk. score	0 wk. score	41	324.20	415.76	44	316.75	420.89
2	Step test	6th wk. score	0 wk. score	41	364.73	413.90	44	370.36	420.89
3	Step test	Intersect 10th wk.	Intersect 0 wk.	41	326.02	414.91	43	323.36	425.94
4	Physical fitness	10th wk. score	0 wk. score	40	330.33	175.33	44	340.07	164.50
5	Physical fitness	6th wk. score	0 wk. score	39	284.54	177.36	41	274.10	160.51
6	Physical fitness	Intersect 10th wk.	0 or 1st wk. score	40	334.85	195.75	44	338.48	178.77
7	Pull-ups	10th wk. score	0 wk. score	40	8.55	5.83	44	8.73	5.27
8	Sit-ups	10th wk. score	0 wk. score	41	53.27	36.61	44	51.14	33.59
9	Push-ups	10th wk. score	0 wk. score	40	32.58	19.58	44	35.75	19.70
10	Grip strength	10th wk. score	0 wk. score	42	48.83	49.43	44	48.70	47.59
11	Contest march	Minutes	Squat jumps	42	129.07	33.48	42	126.36	34.38
12	Outdoor cold	Temp. drop	Body fat, %	39	1.57	7.08	39	1.33	7.61
13	Indoor cold	Change in temp.	Body fat, %	37	−0.2297	7.13	39	+0.1769	8.05
14*	Body weight	10th wk.	0 wk.	42	66.31	70.40	42	66.69	71.89
15	Body weight	7th wk.	0 wk.	42	70.22	70.40	43	70.87	72.04
16	Body fat, %	10th wk.	1st wk.	42	3.47	6.93	44	3.35	7.76
17	Eosinophils	% of control, FM	% of control, basal	42	36.14	112.17	44	30.71	94.95
18	Eosinophils	% of control, FM	1st count	39	35.93	198.38	41	31.50	166.61

* Analyses 14-18 are fully discussed in Part II of this communication.

TABLE XI (*Contd.*)

Analysis no.	Means of variates adjusted for covariance		Difference between adjusted means, control minus supplemented						Coefficient of regression of variate on covariate			
			Diff., a	Standard error	F ratio	P	95% confidence limits L_1	L_2	Regression co-efficient, γ	Standard error	t	P
	Control	Suppl.										
1	324.97	315.71	9.26	8.14	1.295	0.23	+25.45	−6.93	0.4329	0.1127	3.841	0.001
2	366.52	368.76	−2.24	7.64	0.086	0.77	+12.96	−17.44	0.4284	0.1056	4.057	0.001
3	328.78	320.54	8.24	8.07	1.076	0.29	+24.30	−7.81	0.5406	0.1897	2.850	0.01
4	326.75	344.11	−17.36	12.05	2.075	0.17	+6.62	−41.32	0.7563	0.1102	6.863	0.001
5	277.51	280.87	−3.36	9.92	0.115	0.74	+16.37	−23.11	0.8939	0.0909	9.834	0.001
6	329.12	344.38	−15.26	10.97	1.938	0.18	+6.56	−37.09	0.7525	0.0925	8.135	0.001
7	8.33	8.95	−0.62	0.55	1.282	0.18	+0.47	−1.71	0.9336	0.0942	9.911	0.001
8	52.53	51.81	0.72	2.20	0.106	0.75	+5.09	−3.65	0.4715	0.1178	4.003	0.001
9	32.68	35.80	−3.12	2.18	2.044	0.17	+1.22	−7.46	0.8995	0.1844	4.878	0.001
10	47.94	49.58	−1.64	1.05	2.453	0.13	+0.44	−3.73	0.9049	0.0675	13.406	0.001
11	130.43	128.01	2.42	3.54	0.471	0.49	+10.69	−3.39	−0.5637	0.1473	3.827	0.001
12	−1.56	−1.34	0.22	0.11	3.834	0.052	+0.44	+0.00	−0.0510	0.0138	3.711	0.001
13	−0.184	0.226	−0.42	0.14	8.598	0.007	−0.14	−0.68	0.0134	0.0179	0.750	0.45
14	66.88	66.12	0.76	0.35	4.741	0.04	+1.46	+0.07	0.7717	0.0178	43.403	0.001
15	70.91	70.19	0.72	0.42	2.953	0.08	+1.54	+0.11	0.8421	0.0213	39.517	0.001
16	3.64	3.18	0.46	0.22	4.732	0.04	+0.90	+0.04	0.3975	0.0262	15.166	0.001
17	36.45	30.27	6.18	2.38	6.721	0.02	+10.92	1.44	−0.0315	0.0277	1.135	0.25
18	36.06	31.26	4.80	2.41	3.968	0.05	+9.58	0.00	0.0095	0.0152	0.624	0.55

week as the variate and the intersect with the first week as the covariate (Table XI, Analysis 3). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was 8.24, the control group having the higher pulse sum. The 95 per cent confidence limits of this difference are +24.30 and −7.81. The F ratio for the difference was 1.076, giving a P value of 0.29 which is not statistically significant.

Army Physical Fitness Test

The indoor alternative of the Army physical fitness test[10] as used in this study consists of the following events (the aspects of physical fitness which each is designed to measure were outlined by Wieman[11]):

Pull-ups—To measure strength and endurance of arm and shoulder flexor muscles.

Squat-jumps—To measure strength and endurance of leg muscles.

Push-ups—To measure strength and endurance of arm and shoulder extensor muscles.

Sit-ups—To measure strength and endurance of abdominal muscles.

Squat-thrusts—To measure agility and co-ordination.

During the present experiment these events were administered in the above order to all subjects at approximately weekly intervals for a total of eleven tests, excepting times when a man was unable to perform due to illness or injury.

Procedure. Subjects performed tests in long underwear and socks. At least an hour was allowed after breakfast before testing was performed. Team-mates counted the number of performances of each event. Several physicians who administered the tests monitored the form and frequently checked the accuracy of performance counts. Significant discrepancies of count were unusual.

Requirements of good form were reiterated before each test administration. Monitoring physicians called the attention of subjects to infractions of form, but men were given credit for events performed prior to being thus corrected.

One platoon was tested at a time. Events were performed in squads. All squads of the platoon performed each event in succession before proceeding to the next event. Thus, each man was able to rest for five to ten minutes between events. The entire company was usually tested within a period of about four hours.

Scoring. A standard method of scoring designed to give equal weight to each event is presented in the Army Manual of Physical Training.[10] It is stated, "The data from which these scoring tables were derived were based upon the performance of troops in good physical condition. The mean or average score is 50 points and the range is from 0 to 100 points. Thus a score of 50 represents the average score of individuals in good physical condition. Not more than 1 per cent of a well-conditioned unit will score above 100.

TABLE XII

Summary of Army Physical Fitness Test Results

Phase of study →			Control	Equilibration			High intake, high output			Rest	Low intake, high output		
Test number →			1	2	3	4	5	6	7	8	9	10	11
Days after capsules started →			−2	0 (III, IV) +2 (I, II)	+9	+16	+21	+26	+33	+40	+47	+54	+61
Number of men tested	Control		36	38	40	40	41	40	39	42	41	38	39
	Suppl.		41	40	43	43	43	43	42	41	44	44	43
Total score on Army P. F. test	Control	Mean	184.6	187.4	198.6	245.3	256.5	283.2	286.3	313.1	323.9	322.4	332.4
		S. D.	59.1	71.2	70.8	77.6	71.9	69.8	72.4	69.6	82.5	92.4	75.6
	Suppl.	Mean	164.1	145.3	178.7	210.6	250.4	246.0	271.1	300.2	323.4	329.2	340.0
		S. D.	64.3	68.3	69.6	79.4	61.2	55.5	62.3	68.6	67.5	91.7	65.5
Pull-ups (max. = 20)	Control	Mean	6.0	6.0	7.1	7.3	7.4	7.4	7.7	7.7	8.4	8.2	8.4
		% max.	0	0	0	0	0	0	0	0	0	0	0
	Suppl.	Mean	5.2	4.7	5.4	5.6	6.9	6.3	6.3	7.3	8.1	8.4	8.8
		% max.	0	0	0	0	0	0	0	2	0	0	0
Squat jumps (max. = 75)	Control	Mean	35.7	33.3	40.1	42.1	48.3	52.9	54.8	59.2	60.4	6.12	62.8
		% max.	0	0	5	10	20	35	41	48	49	55	51
	Suppl.	Mean	35.9	28.0	38.2	41.7	49.6	52.7	56.9	61.4	63.3	60.6	65.1
		% max.	2	0	5	9	21	33	51	52	57	57	58
Push-ups (max. = 54)	Control	Mean	19.8	20.7	21.2	24.6	25.6	27.8	27.7	30.4	31.2	32.0	32.6
		% max.	0	0	0	0	0	0	3	5	2	5	8
	Suppl.	Mean	19.5	19.3	21.9	23.7	26.2	28.7	27.9	28.8	32.5	34.0	35.2
		% max.	0	0	0	0	2	2	2	2	7	14	16
Sit-ups (max. = 79)	Control	Mean	37.6	35.0	35.0	42.0	43.9	48.9	47.8	52.1	52.4	53.3	53.1
		% max.	0	0	0	0	0	0	3	0	2	3	3
	Suppl.	Mean	33.4	26.8	30.5	40.0	41.2	44.4	45.7	47.9	50.4	51.9	51.1
		% max.	0	0	0	0	0	0	0	0	0	0	0
Squat-thrusts (max. = 41)	Control	Mean	24.3	26.7	26.7	30.6	29.4	32.8	31.7	33.2	34.3	34.5	34.9
		% max.	0	0	0	0	0	2	5	10	20	18	23
	Suppl.	Mean	24.0	25.0	25.8	28.6	29.3	29.9	30.7	31.9	33.8	33.8	34.9
		% max.	0	0	0	2	5	0	0	7	16	14	28

Suppl. = Supplemented. % max. = Percentage of subjects achieving maximal scoring credit.

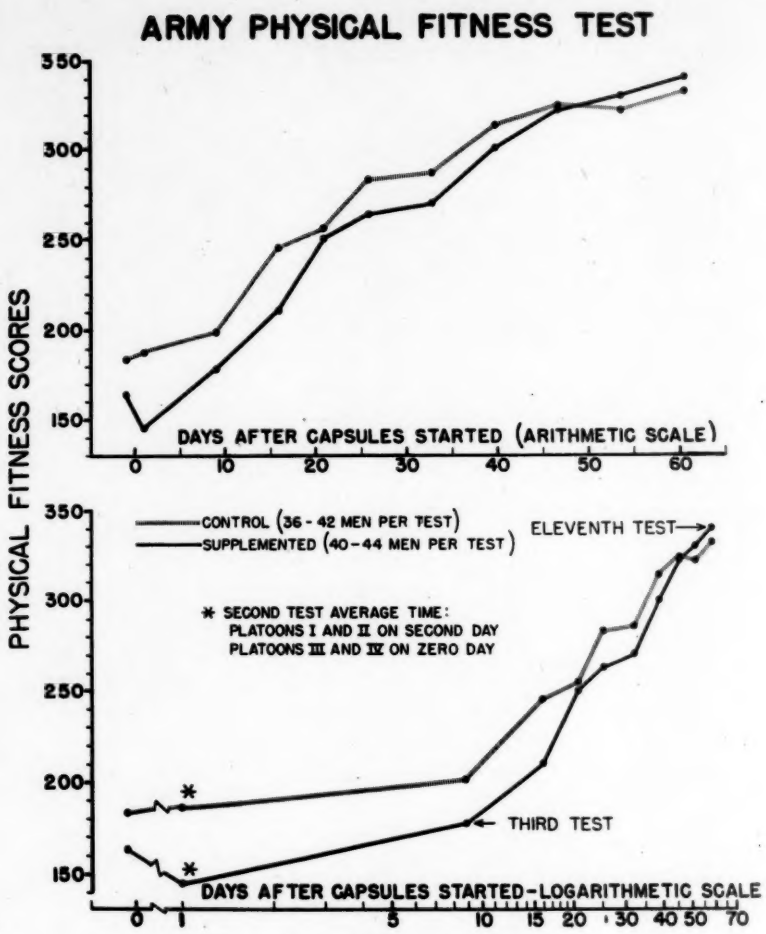

Fig. 6. Army physical fitness test data.

Not more than 1 per cent of a well-conditioned unit fails to score at all." The total score is the sum of each such score for the five events, giving a maximum possible score of 500, and an average score for individuals in good physical condition of 250 points.

Unfortunately, the recommended tables give scores only to 100 for each event. Thus, if a significant number of men achieve the number of repetitions necessary for maximum score, there is no method of differentiating them. As noted in Table XII, many men in the present study were able to achieve maximum scores in certain events, particularly squat-jumps, in which more than half the men achieved maxima toward the end. On the other hand, men sometimes performed an event fewer times than indicated for the minimum score. All such instances were scored as zero regardless of how near or far performance was from the minimum.

The artificial truncation of maximal scores leads to a plateau effect on total scores in those individuals achieving the maximum of individual events. Subsequent improvement in ability to perform that event is not registered. However, the physical-sparing effects of stopping before exhaustion after completing the maximum number of an event might aid in the performance of subsequent events.

Analysis of Results. The trends of total Army physical fitness scores as well as number of pull-ups, push-ups, and sit-ups is indicated for successive tests in Table XII and Figures 6 and 7. It is noted that the mean improvement continued throughout the period of the study, though values tended to approach an asymptote toward the end. When the total scores are plotted against a logarithmic time basis, as noted in Figure 6, the values for the third through the eleventh tests tend to fall along a straight line.

An analysis of covariance was carried out using the score of the last test as the variate and the score of the first test as the covariate (Table XI, Analysis 4). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was −17.36 points, with the supplemented group having the higher value. If this difference in score were evenly distributed over the 5 events of the physical fitness test, it would represent an advantage of the supplemented group over the control equivalent to approximately 1 pull-up, 3 squat-jumps, 2 push-ups, 2 sit-ups, and 1 squat-thrust. The 95 per cent confidence limits of this difference are +6.62 and −41.32 points. The F ratio for this difference was 2.075, giving a P value of 0.17, which, again, was without statistical significance.

Another analysis of covariance was carried out using the score of the seventh test (end of high calorie, high activity period) as the variate and the score of the first test as the covariate (Table XI, Analysis 5). The difference (control minus supplemented) between the means of the two treatment groups adjusted for convariance was −3.36 points, with the supplemented group having the higher value. The 95 per cent confidence limits of this difference was +16.37 and −23.11. The F ratio for this difference was 0.115, giving a P value of 0.74 (not statistically significant).

Individual men showed fluctuations in total test scores unrelated to long-time trends. This was also noted with step-test results. As with the step test, it would seem desirable to increase the precision of measurement by incorporating data gathered from intermediate tests.

It was previously pointed out that a logarithmic slope closely approximated the mean values for the third through the eleventh tests. The probable average if several tests were done on the week of the eleventh test (assuming that they would not serially affect one another) can be estimated by the intersect of a logarithmic slope calculated statistically from values of the third through eleventh test with the time of the eleventh test. This intersect appears to be a suitable variate.

An analysis of covarianoe was carried out using the

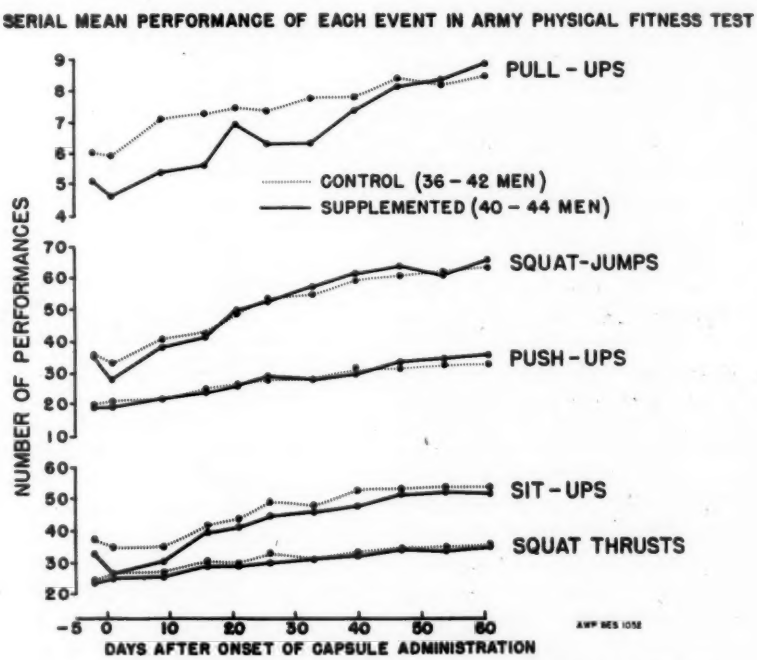

Fig. 7. Mean performance in each event of the Army physical fitness test.

intersect of the log slope of each man with the tenth week as the variate and the score of the first or second test as the covariate (Table XI, Analysis 6). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was −15.26 points, with the supplemented group having the higher value. The 95 per cent confidence limits of this difference are +6.56 and −37.09 points. The *F* ratio for this difference was 1.938, giving a *P* value of 0.18 (not statistically significant).

Those individual events in which only a small percentage of men achieved maximal scores were analyzed in a similar fashion. An analysis of covariance was carried out on the values from these individual events of the physical fitness test using the number of performances in the last test as the variate and the number of performances in the first test as covariate (Table XI, Analyses 7, 8, and 9). The differences (control minus supplemented) between the two treatment groups adjusted for covariance were −0.62 for pull-ups, 0.72 for sit-ups, and −3.12 for push-ups, the supplemented group having the higher values for pull-ups and push-ups, the control group having the higher value for sit-ups. The *F* ratios of these differences were 1.282 for pull-ups, 0.106 for sit-ups and 2.044 for push-ups. None of these *F* ratios are statistically significant.

Hand Dynamometer

Tests were performed at approximately weekly intervals immediately preceding Harvard step tests for a total of 11 times, with a few individual exceptions.

Procedure. A Stoelting hand dynamometer* with scale from zero to 90 Kg. was used. Each subject would dry his hands and take three successive grips on the dynamometer. The greatest of these, usually the first, was taken as the score. Right or left hand was used depending on the handedness of the individual. The reading in Kg. was taken as the score.

Analysis of Results. As noted in Table XIII, there is no particular time trend, and no notable difference between the two treatment groups.

An analysis of covariance was carried out using the score of the last grip strength test as the variate and the score of the first test as the covariate (Table XI, Analysis 10). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was −1.64 Kg.,

* C. H. Stoelting Company, 424 Homan Avenue, Chicago, Ill.

TABLE XIII

Summary of Grip Strength Results

Phase of study →			Control	Equilibration			High intake, high output			Rest and rehab.	Low intake, high output		
Test number →			1	2	3	4	5	6	7	8	9	10	11
Days after capsules started →			−1	+3	+10	+17	+22	+27	+34	+41	+48	+55	+62
Number of men tested	Control		41	40	41	40	42	38	40	42	41	40	42
	Supplemented		44	43	42	43	41	43	43	44	44	44	44
Grip strength, Kg.	Control	Mean	49.6	49.5	49.9	50.1	50.9	49.8	51.4	48.6	48.5	48.0	48 9
		S. D.	7.4	7.4	8.2	8.2	9.9	8.7	8.3	8.1	7.6	8.4	8 4
	Suppl.	Mean	47.1	49.1	48.7	50.0	49.1	49.4	49.3	48.6	49.0	46 6	48.7
		S. D.	9.9	8.1	8.8	7.8	7.3	7 9	8.7	9.8	9.1	10.0	9.8

with the supplemented group having the higher value. The 95 per cent confidence limits of this difference are +0.44 and −3.73. The F ratio of this difference was 2.453, giving a P value of 0.13, which is not statistically significant.

Forced Marches

Procedure and Purpose: Forced marches were designed for two purposes: to require prolonged high energy output with resulting physical stress; and to provide another measure of physical performance, particularly endurance.

Outline of Method: Marches were conducted at quick time with intervals at double time and 10 minutes rest in each hour. One or two platoons marched on a given day, followed by the ambulance and other vehicles as necessary to transport men who dropped out. Time intervals were controlled by stopwatches. Forced marches were performed by all platoons at weekly intervals, except for the rest weeks (weeks 6 and 10). With minor exceptions, the marches for all four platoons in a given week were conducted under the same rules; however, a number of changes were made from week to week. Observations of temperature, wind velocity, and wind direction were made once or twice an hour throughout the marches. In some cases, biochemical studies were performed by drawing blood before and after the march and collecting urine concurrently in plastic bottles carried by the test subjects. These studies will be discussed elsewhere. Coffee without cream or sugar was served at least once during most of the marches, but no food was available. Where meals were missed, the food of the missed meal was offered when the men returned or at the supper meal.

March routes were designed to permit acquisition of data on dropout of a previously determined fraction of each platoon. The marches were terminated for each platoon when this fraction of dropouts had occurred, except in a few instances when platoons reached the objective (camp) before the specified percentage dropped out of the march.

Quick Time and Double Time: In the first week the march was conducted at quick time for the first 40 minutes of each hour, followed by 10 minutes of double time and 10 minutes of rest during the remainder of the hour. The 10 minute period of double time was deemed too long, as it caused lagging of men who could otherwise continue the march for a long period, and also introduced the additional factor of oxygen debt. Therefore, in all subsequent marches, except the last week of the test, a cycle was employed of 8 minutes of quick time and 2 minutes of double time, with five such repetitions during 50 minutes, and, finally, a 10-minute rest period in each hour. Occasionally, over icy sections of road, the double time was omitted. In the final week double time was omitted altogether, with 50 minutes of quick time and 10 minutes of rest in each hour.

Rate of March: In marches 1 through 3, the rate was left up to the platoon leaders, who generally accomplished about 3 miles in each 50 minutes. In the third march, the pace was slowed by the platoon leaders to about 2.7 mi./50 min., with the result that the march lasted until after dark and that the double time pace was so slow that some men kept up without jogging.

In march 4 a rate of 3.5 mi./50 min. was ordered. The rate accomplished was about 3.3 mi./50 min. and the length of the march was substantially shortened before the required number of dropouts occurred.

In marches 5–7 a compromise was made in order to provide a longer period of physical stress: the pace was set at 3.0 to 3.2 mi./50 min.

Dropout Fraction: In marches 1 through 3 the fraction of dropouts to be achieved before termination was 33 per cent. To provide data on more men, this goal was increased to 50 per cent in march 4 and to 75 per cent in marches 5–7. As mentioned above, the march was terminated when a platoon reached the objective (camp) before the specified number of men dropped out.

Routes: All march routes were planned so as to take maximum advantage of the prevailing west or southwest winds as headwinds and to provide the severest and most prolonged upgrades available in the vicinity. Several

of the routes had total gains in elevation of 2200 feet, with almost continuous upgrades for distances of 11 to 20 miles. These grades averaged about 2 per cent in severity, with short stretches as steep as 15 per cent. Except for the last three weeks, the marches were conducted on a network of country dirt roads. During the last three weeks these routes were closed by snowdrifts, and the marches were conducted along the margin of U. S. Highway 30, with adequate traffic control.

Uniform: Since the wind velocity was usually considerably higher along the march routes than at camp, the windchill during marches exceeded that recorded at camp, where the type of uniform was determined. Thus the men were dressed in relatively less adequate clothing for the forced marches than for their other activities. However, because of the high level of exertion, the test subjects were usually not uncomfortably cold during actual marching. They often did become cold during the 10 minutes of rest and while in trucks going to the initial point of the march, or after dropping out.

Scoring: Test subjects were excused from forced marches only for sufficient medical reasons. Febrile illnesses and moderately severe injuries or orthopedic disorders of the lower extremities were the only conditions for which men were eliminated. In a few cases, men with disabilities of a nature which might be expected to interfere with performance were sent on the march but not scored officially, whether or not they completed the march. This was done to give the men the physical training and stress of the march in so far as possible. The decision not to include these individuals in the scoring was in all cases made by the medical officers prior to the start of the march. Test subjects were not informed of these decisions.

Dropout Time: In the first march men were allowed to lag behind the main body of the platoon for considerable distances (up to 200 yards) and were not registered as dropouts until they gave up the effort to keep up. In all succeeding marches men were considered to be dropouts when they lagged more than 20 yards behind the main body and were unable to regain the distance when requested to do so. Those men who stopped voluntarily were allowed to drop out if they had an obvious injury to the legs or feet, appeared exhausted, or were vomiting. Otherwise, they were asked to keep going as long as possible.

In marches 1 to 4 the dropouts were carried in the ambulance. In marches 5 and 6, to remove the stimulus of riding in the heated ambulance, the dropouts were transported in unheated trucks unless they appeared so exhausted that this was considered dangerous. In march 7, to remove altogether the incentive of receiving vehicular transportation, all dropouts who were not seriously incapacitated were marched over the entire route in a rear platoon.

Results. Probably the index of performance of a unit which is of most significance to the Armed Forces is a summation of the rate of effectiveness, i.e., the number of men capable of participating in an activity, plus the performance output of the individual soldier. In this study of forced marches both factors come into play in interpreting the results. The effectiveness rate is the number of men scored in each march, yet those men who went on the march, but were not scored, were more effective than those who did not go at all. The performance output can be measured by the proportion of a march completed by a given subject. However, this is complicated by the fact that only a few men actually dropped out because of complete exhaustion; some dropped out because of traumatic conditions and many more for psychological reasons.

It has not been possible to devise an integrated scoring system which would take into account all of the above contributions, giving each its proper weight. Therefore, it has been necessary to study the factors individually and several of these evaluations will be presented.

A summary of results in the forced marches is given in Table XIV.

Wilcoxon Ranking Test. The analysis of the forced marches presents certain difficulties on account of the fact that the data do not have a Gaussian distribution. The times and

TABLE XIV
Summary of Forced Marches

Date	Platoon	Wind chill (mean)	Total time	Total distance	Miles per Hour	No. men marching			Dropouts		
						Total	Control	Supplemented	Total	Control	Supplemented
			Min.	*Miles*							
12J	I	800	205	10.5	3.1	23	11	12	9	6	3
13J	II	900	260	13.5	3.1	18	9	9	6	2	4
14J	III	600	315	14.7	2.8	20	9	11	7	3	4
15J	IV	1300	153	7.3	2.9	21	10	11	9	4	5
21J	I	1000	210	12.1	3.5	20	10	10	9	4	5
21J	II	1000	347	17.3	3.0	20	11	9	7	4	3
22J	III	1050	425	21.0	3.0	19	8	11	6	2	4
22J	IV	1050	412	20.9	3.0	20	10	10	7	3	4
31J	I	950	571	25.1	2.6	21	10	11	4	2	2
30J	II	950	536	25.1	2.8	21	11	10	4	3	1
30J	III	950	492	23.0	2.8	20	9	11	7	3	4
31J	IV	950	449	20.0	2.7	20	10	10	11	5	6
4F	I	700	236	13.4	3.35	20	9	11	13	5	8
5F	II	950	313	17.3	3.33	20	10	10	11	5	6
6F	III	850	318	17.6	3.32	19	8	11	10	5	5
7F	IV	1000	322	16.8	3.11	19	9	10	10	5	5
20F	I	1450	310	15.6	3.0	20	8	12	11	3	8
21F	II	1400	518	22.1	2.6	20	10	10	4	2	2
20F	III	1450	310	15.6	3.0	19	9	10	11	6	5
21F	IV	1400	518	22.1	2.6	21	10	11	15	7	8
27F	I	950	460	25.1	3.3	21	10	11	13	5	8
27F	II	950	275	15.1	3.3	21	11	10	16	7	9
28F	III	900	494	25.5	3.1	19	8	11	14	8	6
28F	IV	900	494	25.5	3.1	21	10	11	15	6	9
6M	I	975	495	25.1	3.04	22	10	12	8	6	2
7M	II	925	483	25.1	3.1	21	11	10	1	1	0
6M	III	975	495	25.1	3.04	21	10	11	6	4	2
7M	IV	925	483	25.1	3.1	20	10	10	3	2	1
					TOTALS	567	271	296	247	118	129

distances that each man went were recorded, but the marches were terminated when 30 to 70 per cent of the men had fallen out. For the remainder of the men, therefore, we have no idea of how much further they would have gone, so there is the further difficulty that the data are truncated.

A method of approach which avoids the assumption of normality is to rank the men serially in order of the distance that they went. There will be a number of ties, in which two or more men fall out simultaneously. To each of these sets of ties a sufficient number of rank numbers to equal the number of men tieing are allocated, and then each man in the set is given the mean of the group of ranks. This same procedure can be applied to the group who had not fallen out at the termination of the march, and, also, in the case where dropouts from all causes are considered and there is a group of men who did not even start the march, to the non-starter.

It is clear that if men fall into two groups classified according to their vitamin supplementation, and if the supplementation is effective in the sense that the men receiving it tend to go farther in the march than those not receiving it, the supplemented group will be displaced relative to the unsupplemented so that their mean rank is higher.

The Wilcoxon ranking method[12,13] was used for testing the significance of the difference between mean ranks. The sixth forced march (Feb. 27–28) was selected to be analyzed by this method because in this march a high percentage of dropouts was achieved and

TABLE XV
Change in Performance on Forced Marches

Change	Supplemented		Control		Total	
	No.	%	No.	%	No.	%
Deterioration	22	50.0	17	47.2	39	48.8
No change	12	27.3	14	38.9	26	32.5
Improvement	10	22.7	5	13.9	15	18.8
TOTAL	44	100.0	36	100.0	80	100.1

$X^2(2) = 1.68$; $X^2_{0.50}(2) = 1.39$; $X^2_{0.75}(2) = 2.77$.
$X^2_\epsilon(2)$ is the ϵth percentile on the X^2 distribution for 2 degrees of freedom.

at this time the subjects had been on the supplementation regime for a long period. Two separate analyses were carried out, one in which failure to complete the march for any reason, including failure to participate in the march, was tabulated, and the second analysis in which only those men who entered the march were considered and only dropouts for reasons other than specific injuries to the legs and feet were tabulated. In neither analysis was there any indication that the supplemented group performed significantly better than the control group.

Change in Proportion of Marches Completed. This study included data only on men dropping out because of fatigue. A score was obtained for each man in each march based upon the proportion of the march which he completed. In order to differentiate between men who were still going at the termination of the march and the last man or men to drop out, the latter were empirically assigned a proportion of completion of 90 per cent, i.e., the total time of the march was increased by $^1/_9$ over the elapsed time. Actual marching times were then divided by the corrected total time to obtain scores.

A "before" rating was obtained by taking the average of the scores on the first two marches, or the score on either of them in case of missing data. An "after" rating was similarly obtained for the last two marches (6 and 7). The "before" rating was subtracted from the "after" rating to obtain a "change of performance" for each man. These changes were grouped according to whether they showed improvement with time, no change, or deterioration. The results, which are presented in Table XV, indicate no significant difference between supplemented and control subjects.

Frequency of Dropouts Due to Fatigue. In the supplemented group, 34 men dropped out a total of 86 times because of fatigue; in the control group, 31 men dropped out a total of 69 times for this cause. Exact statistical analysis of these data is difficult, but there is no indication of a treatment effect.

Effectiveness Rate. The numbers of supplemented and control subjects who participated and were scored in all marches and those who missed or were not scored in one or more marches are compared in Table XVI. A

TABLE XVI
Number Participating in All Marches or Missing at Least One March

Number of marches missed	Number of men		
	Supplemented	Control	Total
None	35	23	58
One or More	9	20	29
TOTAL	44	43	87

$X^2(1) = 5.52$; $X^2_{0.025}(1) = 5.02$.

significantly larger incidence of ineffectiveness existed in the untreated group. However, as shown in the section on morbidity records, there was a greater incidence of illness and injuries in the control group, both before and after the beginning of capsule administration.

"Iron Men." A comparison was made between the number of men in the two groups who finished all forced marches from 3 to 7, provided they did not miss more than one of these because of illness. These data are presented in Table XVII. Statistically, there is no significant difference between supplemented and control subjects.

TABLE XVII
"Iron Men" in Forced Marches

Category	Supplemented	Control	Total
Iron	8	6	14
Not iron	36	37	73
TOTAL	44	43	87

X^2 (1) = 0.06 (with continuity correction).
X^2 0.995 (1) = 0.0393.

In Table XVIII, an analysis of dropouts according to certain categories of causes of dropouts is presented. There is no suggestion of a difference between the two treatment groups.

TABLE XVIII
Summary of Dropouts in Forced Marches

Cause of dropout	Control	Supplemented
Fatigue	69	87
GI upset	6	9
Legs (specific injuries)	33	23
Upper respiratory infections	2	4
Quit	6	2
Other	2	4
TOTAL	118	129
No. men marching	271	296
Per cent dropouts	43.5	43.6
No. of men who dropped out of all marches entered	6	3
Average dropout time for men dropping out because of fatigue (hr.)	4.31	4.21

In summary, although an integrated scoring method could not be devised to take account of all factors in the results of the forced marches, several tests on individual factors failed to show a significant effect of treatment.

Comment. Experience with the forced march as a measure of performance, and specifically endurance, demonstrated that the usual cause of dropping out was loss of the will to proceed. It is not proper to call a man a quitter if he stops after marching 20 miles uphill into a fierce wind, yet in only rare instances did men apparently reach the limit of their capacity to march. This points up the fact that factors of motivation enter importantly into measurements of physical endurance.

Study of the summary table of forced march data (Table XIV) suggests that the rate of march was the variable most closely correlated, in inverse manner, with the distance marched. The average windchill during the marches varied from 600 to 1450, i.e., from pleasantly cool to dangerously cold, without a clearcut effect on performance. Reduction of caloric intake in the last two weeks of the experiment likewise did not clearly influence the distance or rate of the march.

CONTEST MARCH

On February 23, 1953, at the beginning of the second week of caloric restriction, a contest march was conducted. This was designed to provide high output work stress and also to give a measure of performance through the motivation of both individual and platoon competition.

The contest march was run over a course of 9.2 miles, being an out and back trip on the road between the camp and Highway 30. The road was covered with packed snow and had many steep grades. Test subjects were permitted to set their own pace, alone or in groups. Platoon scoring was on the basis of mean time for all members of the platoon. Prizes were awarded to the winning individual and the winning platoon.

Motivation of the men to do their best was generally good. Contributing to this was, of course, the spirit of competition and also the fact that no motor vehicles were available to pick up stragglers, so that all were obliged to return to camp. The spread of elapsed times for individuals was large ($1^1/_3$ to 3 hours), giving a good opportunity for evaluation of differences between the treatment groups.

A summary of the results appears in Table XIX. The mean time for all men was slightly over 2.1 hours, signifying a rate of 4.3 mph. The mean difference between the times of control and supplemented groups was about 2.7 minutes favoring the treated group.

An analysis of covariance was carried out using the time in minutes required to complete the march as the variate and the mean number of performances of squat jumps on the first and second physical fitness tests as the covariate (Table XI, Analysis 11). The difference between the means was 2.42 minutes, with

TABLE XIX

Results of Contest March on Feb. 23, 1953, 9.2 Mile Course

Platoon		Control	Supplemented	Total
I	No. men	11	12	23
	Avg. min.	135.83	133.10	134.40
II	No. men	11	10	21
	Avg. min.	125.82	120.53	123.30
III	No. men	10	10	20
	Avg. min.	132.07	123.33	127.70
IV	No. men	10	11	21
	Avg. min.	121.89	126.76	124.44
TOTAL	No. men	42	43	85
	Avg. min.	128.99	126.28	127.6
	Avg. hours	2.150	2.105	2.127
	Mph.	4.28	4.37	4.33

the control group having the higher value. The 95 per cent confidence limits of this difference are +10.69 and −3.39. The F ratio was 0.471, giving a P value of 0.49, which is not statistically significant. The regression of the variate on the covariate was statistically highly significant.

Observations on Factors Other Than Physical Performance

COLD EXPOSURE TESTS

To evaluate the possibilty that response to cold might be altered by supplementation, as well as to increase the overall exposure of the men to the cold, two types of cold experiment were done in this study.

Outdoor Cold Exposure

In the first type, the test subjects were exposed outdoors once a week during the seventh, eighth, and ninth weeks for a period of four hours. During this time they lay upon the ground or on or under the snow in a prescribed uniform. They were allowed only to raise themselves upon one elbow. All other activity, such as clapping the hands together or stamping of feet, was prescribed. Some men were permitted to go to the latrine for a few minutes during the test. Rectal temperatures were taken in the barracks after breakfast, just prior to the period of cold exposure, and immediately after the exposure. The thermometers used had been checked for accuracy by immersion in a constant-temperature bath, discarding those that read more than 0.1° F. from bath temperature. Mineral oil was used to lubricate the thermometers. The thermometers were left in place for a minimum of 3 minutes, accurately timed by stopwatch.

During the first outdoor cold test the men wore full winter uniform with liners in both the parka and in the pants. During the second outdoor test the men wore the entire winter uniform except that the liner in the pants was omitted. In the third test the liners in both the parka and the pants were omitted. During the entire period of all tests for all platoons the average air temperature to which the men were exposed was 20.4° F., with slightly higher values of an average of 27.5° F. immediately above the snow or surface, and 23.4° F. immediately below the snow or surface. The average wind velocity during all of the tests was 15.6 mph., and the average windchill was 1154 cal. per sq. meter per hour. The average relative humidity was 54 per cent. The average total hourly radiation was 58 cal. per sq.cm. Snow was on the ground only during the first two of the five days when the cold tests were conducted, and on these days only a few of the men were on the snow or under it. However, there was approximately one inch of snow on the average for all of the outdoor tests.

In the course of the three outdoor cold exposures, four men were excused because of illness from one test each. In addition, eight men dropped out of one of the three tests because of severe complaints of coldness.

TABLE XX

Pooled Mean Values for Rectal Temperature Falls in 3 Outdoor Cold Exposure Tests

Platoon	Control		Supplemented		Difference between control and supplemented groups
	No. of men	Mean fall in rectal temp., ° F.	No. of men	Mean fall in rectal temp., ° F.	
I	10	1.56	12	1.34	0.22
II	10	1.66	9	1.52	0.14
III	8	1.64	9	1.31	0.33
IV	11	1.46	9	1.16	0.30

Adjusted mean difference between treatment groups = 0.22° F. $F = 3.834$. $P < 0.05$.

TABLE XXI
Means and Standard Deviation of Initial and Final Rectal Temperature in Three Outdoor Cold Tests (° F.)

		Control		Supplemented	
		Initial	Final	Initial	Final
Feb. 22	No. of men	39	39	42	42
	Mean	98.915	97.580	98.936	97.829
	S. D.	0.346	0.666	0.416	0.541
Feb. 25–26	No. of men	41	41	44	44
	Mean	98.907	97.317	98.825	97.491
	S. D.	0.407	0.668	0.748	0.556
Mar. 4–5	No. of men	38	38	42	42
	Mean	98.534	96.716	98.624	97.041
	S. D.	0.407	0.828	0.368	0.726

These eight men were equally distributed between the control and supplemented groups.

At the end of the 4-hour period of exposure to the cold, the men were taken into an unheated room and the rectal temperatures again taken. The results are summarized in Tables XX and XXI. The control group showed a mean drop of 1.57° F. and the supplemented group a mean drop of 1.33° F.

An analysis of covariance was carried out using the mean fall in rectal temperature in degrees F. for each man for the three outdoor cold tests as the variate and the per cent body fat as determined from skin-fold measurements at the time of the first anthropometric observations as the covariate (Table XI, Analysis 12). The difference (control minus supplemented) between the means of the two treatment groups adjusted for covariance was 0.22° F., with the supplemented group showing the smaller fall in rectal temperature. The 95 per cent confidence limits of this difference are 0.44 and 0.00. The *F* ratio of the difference was 3.834, giving a *P* value of 0.05, indicating statistical significance at the 5 per cent level of probability. The regression of fall in rectal temperature on per cent body fat was statistically highly significant.

Indoor Cold Exposure

The second type of cold test was conducted in an unheated room in one of the temporary buildings. The fire in the room was put out on the night before the test and no heating was done during the tests. The temperatures recorded in the room during the tests ranged from 0 to 38° F. After obtaining the rectal temperature of each man while still in his sleeping bag in the barracks, the men arose and wore a standard uniform consisting of fatigue jacket and trousers without underwear, and low quarter shoes with socks. Breakfast was withheld until after the cold exposure test. Rectal temperatures were obtained on each man every twenty minutes during the 2 hours of exposure. There was essentially no wind in the room, and the men were either standing or sitting on benches. The results for all four platoons are shown in Figure 8 and Table XXII. The supplemented group showed a smaller mean fall in rectal temperature.

It will be noted that the rectal temperature showed a considerable rise during the first 20 minutes of exposure. This rise can be attributed to a combination of two factors: (1) the rise in temperature which accompanies arising from sleep, and (2) the transient rise in rectal temperature which occurs on acute exposure to cold.[14] Following this rise, a gradual fall in rectal temperature occurred, which did not always reach the basal temperature taken while the men were in their sleeping bags, so that the mean change in temperature was positive in some instances.

An analysis of covariance was carried out using the change in rectal temperature in degrees F. (final minus initial rectal temperature) as the variate and the per cent body fat as determined by skin-fold measurements as the covariate (Table XI, Analysis 13). The difference (control minus supplemented) between the two treatment groups adjusted for covariance was −0.42° F., the control group showing an adjusted mean fall in rectal temperature of 0.184° F., and the supplemented group an adjusted mean rise of 0.226° F. The 95 per cent confidence limits of this difference are −0.14 and −0.68° F. The *F* ratio of the difference was 8.598, giving a *P* value of 0.007, indicating high statistical significance at the 0.7 per cent level of probability. The regression of change in rectal temperature on per

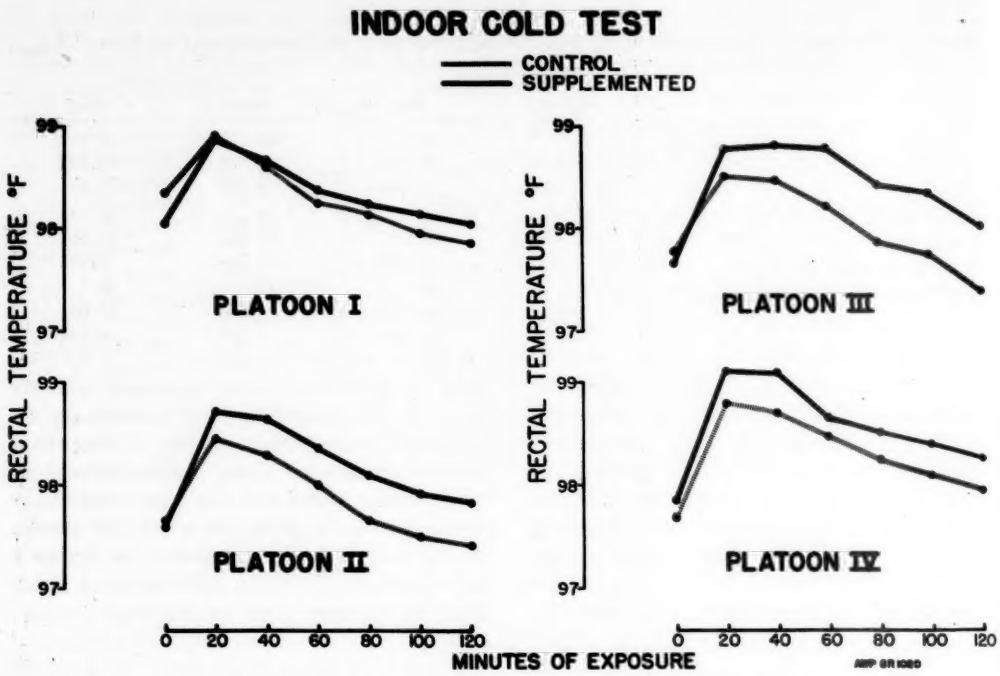

Fig. 8. Mean rectal temperature during indoor cold exposure tests.

TABLE XXII

Results of Indoor Cold Exposure Tests

Platoon	Range of room temp., ° F.	Control		Supplemented		Difference, control minus supplemented, ° F.
		No. of men	Mean temp. change ° F.	No. of men	Mean temp. change, ° F.	
I	27–38	11	−0.4909	12	−0.0250	−0.4659
II	21–25	11	−0.2273	10	+0.1900	−0.4173
III	0–10	7	−0.3714	9	+0.2667	−0.6381
IV	3–8	8	+0.2500	8	+0.3625	−0.1125

Weighted mean difference between control and supplemented, −0.41° F. $F = 8.598$. $P < 0.01$.

A positive value signifies a rise in rectal temperature, a negative value a fall. In the difference column, the negative signs indicate that the fall in temperature was greater or the rise was smaller, in the control than in the supplemented group.

cent body fat was not statistically significant. It is interesting to note that this regression was significant in the outdoor cold test.

PERFORMANCE TESTS DURING COLD EXPOSURE

On one of the days of outdoor cold exposure a set of simple tests of psychomotor performance were performed. The subtests of the Macquarrie Test for Mechanical Ability known as the Tracing, Tapping, and Dotting tests were used. The test is published by the California Test Bureau, 5916 Hollywood Blvd., Los Angeles 28, Calif. The tests were administered in an unheated building, with the men sitting on benches and using boards on their knees as writing surfaces. Administration of the test required about 5 minutes and the men returned promptly to the outside of the building after each testing. On the first day (March 4) members of platoons I and II were subjects and the tests were given just before

exposure, and after 75, 155, and 215 minutes of exposure to cold. There was a tendency for improvement in performance in 2 of the subtests (Tracing and Tapping) in spite of exposure to cold. It was considered plausible that this improvement was due to a practice effect which was stronger than any deleterious effect from cold exposure which might be operating. Therefore on the next day (March 5) when Platoons III and IV were tested the tests were given only twice to each man, once immediately before cold exposure and again after 4 hours of exposure. The results showed that under these conditions deterioration of performance occurred in each subtest. Statistical analysis was performed to determine whether the difference between the change in performance of the control and supplemented groups was significant. None of these differences approached statistical significance.

Discussion

In any experiment, deductions must be related to the conditions of the experiment and to the types of observations performed. In a broad study such as this one, a large number of alternatives could have been utilized in many of the conditions of the experiment and many of the types of observations. Such alterations might conceivably have led to quite different conclusions. For this reason all of the evaluations stated here are subject to the qualification that they apply "under the conditions of this experiment."

The major stresses to which the test subjects in this experiment were subjected were high physical activity, exposure to cold, and, during the final three weeks, caloric restriction. Exposure to the altitude of the campsite (8310 feet) may be considered an additional stress. Measurements of physical performance were the major criteria by which the ability to withstand these stresses was evaluated. The high physical activity and the exposure to altitude were accomplished in the expected fashion. However, the exposure to cold presented some problems. It soon became apparent that under the prevailing weather conditions, even with an average windchill of 1000 Kg. cal./sq.m./hr., which is regarded as "very cold," the program of high physical activity largely prevented the men from becoming uncomfortably cold during the time that they were active. However, the men were not engaged in high physical activity every moment while out of doors, and during the periods of low activity they did become cold. In a sense, the conditions resembled those that would be encountered by troops on active maneuvers in a cold environment.

All of the tests of physical performance except the forced marches were conducted in buildings at comfortable temperatures. During the forced marches the men were generally not uncomfortably cold except during the rest periods and while being transported to or from the march in trucks. It was realized at the time the experiment was planned that the indoor performance tests would be conducted in the absence of cold, but it was felt that the cold exposure at other times might exert an effect on the performance of these tests. We have been unable to find information in the literature on whether chronic exposure to cold has an effect on physical performance measured at comfortable temperatures. If it were desired to determine the effect of body cooling *during* high activity, a very cold environment would be required, cold enough to overcome the extra heat production of the exercise.

In planning the experiment, it was anticipated that the degree of caloric deprivation used during the last three weeks of the study would lead to deterioration in physical performance. It was postulated that the degree of deterioration might be different in the two treatment groups, affording a means of assessing the value of supplementation. A search of the literature failed to yield information on the degree of caloric deprivation required to produce impairment of physical performance. In the study of Keys, *et al.*,[15] in which the rate of caloric deprivation was comparable to that of the present study, marked deterioration in physical performance was present at the end of twelve weeks; however, observations were not made at an earlier time during the study and, therefore, the point at which deterioration began cannot be ascertained. In the present study no evidence of impaired perform-

ance was present at the end of the 3-week period of relative caloric insufficiency; in fact, improvement in performance continued to occur. Because of the dearth of information on the effect of caloric deficit on physical performance, this observation is of significance by itself, although it does not bear upon the question of the effect of vitamin supplementation on performance.

Cold Exposure Tests

In addition to the effect which exposure to cold might have on physical performance, it is of interest to consider also the body's reaction to the stress of cold exposure *per se*. The cold exposure tests were performed to give information on this point. The fall in rectal temperature which occurred on exposure to cold was less in the supplemented than in the control group. Although the difference in magnitude of the fall in the two groups was small, it was statistically significant. It is reasonable to assume that the ascorbic acid in the vitamin mixture was responsible for this effect, because Dugal and Fortier[16,17] obtained a similar effect in monkeys supplemented with only ascorbic acid. However, Glickman, *et al.*,[18] failed to find an effect of vitamin supplementation, including ascorbic acid, on fall in rectal temperature during cold exposure in human subjects. The reason for the discrepancy between the results of Glickman, *et al.*, and those in the present study is not apparent. There were a number of differences in the conditions of the two experiments, including the conditions of cold exposure and the dose and kinds of vitamins.

The present study was not designed to obtain information on resistance to cold injury such as frostbite. Dugal and Fortier[16,17] reported that in monkeys receiving ascorbic acid supplementation not only was the fall in rectal temperature less, but also the incidence of frostbite of the tail was less than in the unsupplemented animals. Further studies are required to determine whether an increased resistance to frostbite can be afforded human subjects by vitamin supplementation.

It is of interest to note that we were unable to detect any differences between the two treatment groups in subjective reaction to cold, in shivering, in blanching and erythema of the extremities, or in psychomotor performance tests.

Inasmuch as the present study was directed primarily at studies of physical performance, further studies on the effect of vitamin supplementation on the physiologic and pathologic reactions to cold exposure while at rest would appear to be warranted. Militarily, this set of conditions is of importance in that it simulates the situation in which a man is "pinned down" and held at relative inactivity while exposed to the cold.

Summary

During the period January 2, 1953 to March 10, 1953, an experiment to study the effect of vitamin supplementation on physical performance while residing in a cold climate was performed at Pole Mountain, Wyo. (8300 ft. elevation), utilizing 86 military personnel as test subjects. The test subjects were randomly divided into two groups. The control group (42 men) received a capsule containing 6 mg. of ascorbic acid 4 times a day. The supplemented group (44 men) received 4 times a day capsules identical in size and appearance, except for the color, and these contained 10 mg. thiamine, 10 mg. riboflavin, 100 mg. niacinamide, 80 mg. calcium pantothenate, 40 mg. pyridoxine, 2.5 mg. folic acid, 300 mg. ascorbic acid, and 4 μg. of vitamin B_{12} per capsule. The subjects were maintained on a program of high physical activity, mostly out-of-doors, throughout the test. During the period of the test the average daytime weather conditions were as follows: temperature 26° F., wind velocity 13 mph, windchill 1030 Kg. cal./sq.m./hr. The clothing worn during the outdoor activities was prescribed and was restricted to less than the amount required for comfort when inactive under the prevailing weather conditions. During the first six weeks of the test, 4100 calories were offered in the diet, and a mean of 3500 calories was consumed. During the last three weeks, 2250 calories were offered in the diet, and essentially all of this was consumed. Physical performance was measured at approximately weekly

intervals, using the Harvard step test, Army physical fitness test, hand dynamometer and a standardized forced march technique. A contest march was performed once during the seventh week of the test. In the Harvard step test and the Army physical fitness test, the performance of both groups continued to improve throughout the 10 weeks of study, including the 3-week period of caloric restriction. There was no statistically significant difference between the two groups in the degree of improvement of performance. Likewise, in the hand dynamometer tests, the forced marches, and contest march there were no statistically significant differences in performance between the two groups.

During the last three weeks of the experiment a study was made of the fall in rectal temperature during exposure to cold while at rest. Three such tests were conducted out-of-doors, one indoors. In all instances the supplemented group showed a lesser fall in rectal temperature, and the difference between the two groups was statistically significant.

A caloric deficit of 1200 calories per day for 22 days did not lead to detectable impairment of physical performance.

NOTE: The results of the psychological, biochemical, and other observations will be presented in Part II of this paper, which will appear in the next issue of this JOURNAL.

REFERENCES

1. DWYER, M. C., and SPECTOR, H.: *Tables of Nutritive Value of Ration Items.* Quartermaster Food and Container Institute for the Armed Forces, Research and Development Branch, Military Planning Division, Office of the Quartermaster General, April 1951.
2. MERRILL, A. L., and WATT, B. K.: *Table of Food Composition for the Armed Forces.* Bureau of Human Nutrition and Home Economics. U. S. Dept. of Agriculture, January, 1941.
3. WATT, B. K., and MERRILL, A. L.: *Composition of Foods: Raw, Processed, Prepared.* U. S. Dept. of Agriculture. Agricultural Handbook No. 8, June, 1950.
4. POLLACK, H., and HALPERN, S. L.: *Therapeutic Nutrition with Special Reference to Military Situations.* National Research Council Report, January, 1951.
5. SIPLE, P. A., and PASSEL, C. F.: Measurement of dry atmospheric cooling in subfreezing temperatures. *Proc. Am. Philos. Soc.* 89: 177, 1945.
6. KEYS, A., and BROŽEK, J.: Body fat in adult man. *Physiol. Rev.* 33: 245, 1954.
7. BROUHA, L., GRAYBIEL, A., and HEATH, C. W.: The step test. A simple method of measuring physical fitness for hard muscular work in adult men. *Rev. canad. de biol.* 2: 86, 1943.
8. JOHNSON, R. E., BROUHA, L., and DARLING, R. C.: A test of physical fitness for strenuous exertion. *Rev. canad. de biol.* 1: 491, 1942.
9. CURETON, T. K.: *Physical Fitness Appraisal and Guidance.* C. V. Mosby Co., St. Louis, 1947.
10. *Dept. of the Army Field Manual, Physical Training,* FM 21-20, U. S. Govt. Printing Office, Washington, D. C., 1950.
11. WIEMANN, E. E.: Some results of physical training under the Army Specialized Training Program. *Research Quart.* 16: 87, 1945.
12. WILCOXON, F.: Individual comparison by ranking methods. *Biometrics Bull.* 1: 80, 1945.
13. KREESKAL, W. H., and WALLIS, W. A.: Use of ranks in one-criterion variance analysis. *J. Am. Statist. Assoc.* 47: 583, 1953.
14. BAZETT, H. C., and NEWBURGH, L. H.: *Physiology of Heat Regulation.* W. B. Saunders, Philadelphia, 1949.
15. KEYS, A., BROŽEK, J., HENSCHEL, A., MICKELSEN, O., and TAYLOR, H. L.: *The Biology of Human Starvation,* Univ. of Minnesota Press, Minneapolis, 1950.
16. DUGAL, L. P., and FORTIER, G.: Ascorbic acid and acclimatization to cold in monkeys. *J. Appl. Physiol.* 5: 143, 1952.
17. DUGAL, L. P.: *Acclimatization to Cold Environment. Symposium on Nutrition under Climatic Stress, Surveys of Progress on Military Subsistence Problems.* National Research Council, 1953.
18. GLICKMAN, N., KEETON, R. W., MITCHELL, H. H., and FAHNESTOCK, M. K.: Tolerance of man to cold as affected by dietary modifications: High versus low intake of certain water-soluble vitamins. *Am. J. Physiol.* 146: 538, 1946.

RESUMEN

Efecto de la administración suplementaria de vitaminas en soldados residiendo en un medio ambiente frío. Parte 1. Acción física y respuesta a la exposición al frío.

Durante el período comprendido entre el 2 de Enero y el 10 de Marzo de 1953 se realizó un experimento con el objeto de estudiar el efecto de la administración de vitaminas sobre la actividad física en personas residiendo

en un clima frío. Este estudio se llevó a cabo en la Montaña Pole, Wyoming (8300 piés de altura), utilizando como sujetos de experimentación a 86 indivíduos del servicio militar. Estos sujetos fueron divididos al azar en dos grupos. Cada uno de los del grupo control (42 hombres) recibió una cápsula, 4 veces al día, conteniendo 6 mg. de ácido ascórbico. Los del grupo suplementado (44 hombres) recibieron, 4 veces al día, una cápsula idéntica en tamaño y apariencia, con excepción del color, que contenía 10 mg. de tiamina, 10 mg. de riboflavina, 100 mg. de niacinamida, 80 mg. de pantotenato de calcio, 40 mg. de piridoxina, 2,5 mg. de ácido fólico, 300 mg. de ácido ascórbico y 4 microgramos de vitamina B_{12} por cápsula. Durante el período de experimento los indivíduos fueron mantenidos bajo un programa de actividad física llevada en su mayor parte al aire libre. Las condiciones del tiempo durante el día fueron las siguientes: temperatura: 26°-F, velocidad del viento 13 mph., *windchill* 1030 kg./cal./milla cuadrada/hora. Los vestidos usados durante el período de actividad al aire libre fueron prescritos y restringidos a menos de la cantidad requerida para la comodidad durante el período inactivo en las condiciones climatéricas reinantes. En las seis primeras semanas del experimento se ofreció una dieta conteniendo 4100 calorías, habiéndose consumido 3500 como término medio. En las tres semanas finales se ofreció una dieta conteniendo 2550 calorías y prácticamente toda esta cantidad fué consumida. La capacidad física fué medida aproximadamente con una semana de intervalo, utilizando la "Harvard Step Tests," la prueba de aptitud física del Ejército, el dinamómetro de mano, y marcha forzada con una técnica estandardizada. Durante la séptima semana del experimento se realizó un concurso de marcha. En las pruebas Harvard y de aptitud física del Ejército, la capacidad de ambos grupos continuó mejorando durante las diez semanas del estudio, incluyendo las tres semanas de restricción calórica. No hubo una diferencia estadísticamente significante en el grado de mejoramiento de la capacidad de los dos grupos. Del mismo modo, en las pruebas del dinamómetro de mano, marchas forzadas y en el concurso de marcha, no hubo una diferencia apreciable entre los dos grupos.

Durante las últimas tres semanas del experimento se realizó un estudio de la caída de la temperatura rectal debida a la exposición al frío en estado de reposo. Tres de estas pruebas se llevaron a cabo al aire libre y una dentro de casa. En todos los casos, el grupo suplementado demostró una caída menor de la temperatura rectal y la diferencia entre los dos grupos fué estadísticamente significante.

Un déficit calórico de 1200 calorías por día durante 22 días no perjudicó apreciablemente la capacidad física.

Life is Real . . . ?

"One could argue, in fact, that much that is wrong with us, in this particular day and generation, and in this particular land of ours, derives from the life—liberty—pursuit-of-happiness, homeostatic, generally polyanna attitude that we have, and were born and raised in; that we expect too much good and are too impatient of ill; that we need to have driven in upon us more of the notion that the essence of living is struggle and suffering; that we need a more vigorous and fearless acceptance of the pathological; that pathology is as necessary a part of life as physiology, and dying as necessary as living; that hell and its devils are as real, and as important, as the angels in heaven; that our good Saint George, for all his valor, does not kill all the dragons, and that he will find, as indeed all saints have found, that the powers of evil still crowd upon him, when his arm has become weary and his sword is still."

—D. W. Richards. *Scientific Monthly* 77: 289, 1953.

VITAMINS *in* DERMATOLOGY

EUGENE S. BERESTON, M.D., M.SC. (MED.), F.A.C.P.*

AT THE beginning of this century the concept of "accessory factors" in food was first presented by Hopkins.[1] A few years later Funk[2] applied the name of "Vitamine" to these factors, since he believed that the product isolated from rice polishings was a chemical "amine" and that it preserved life; hence the prefix vita, "life." The idea of avitaminosis or deficiency diseases was also elaborated by Funk.[3]

As work on the vitamins continued, it became apparent that many vitamin deficiencies had cutaneous manifestations which, in turn, led to the use of vitamins in many dermatoses, some for a specific and some for no specific reason. In order to appreciate the extent of vitamin usage in cutaneous diseases, it is necessary critically to review the voluminous literature.

VITAMIN A

The first property of vitamin A to be recognized was its ability to stimulate growth. Later, xerophthalmia (dry eye disease) was shown to be due to a deficiency of this vitamin.[4] It is clear that the skin and mucosa undergo metaplasia in vitamin A deficiency. The skin lesions take the form of a hyperkeratosis with keratinization of the epithelial linings of the hair follicles.[5,6] This, in turn, blocks oil from lubricating the skin, and produces a dry skin, followed by papule formation and, occasionally, folliculitis.

In vitamin A deficiency there are early conjunctival changes; later, night blindness may be detected by dark adaptation tests, and may be followed in six to nine months by the skin changes described above. The plasma vitamin A content falls only after the deficiency has been prolonged.

The therapeutic use of vitamin A in dermatology began almost with the discovery of the vitamin. The first skin diseases in which it was tried were the follicular hypertrophies and dry scaling conditions such as keratosis pilaris, keratosis follicularis (Darier's disease), and pityriasis rubra pilaris. At first, efforts were made to correlate these diseases with low carotene or vitamin A blood levels, but in Darier's disease this was not possible,[7] and in the other diseases of this group correlation was not always possible.

Peck *et al.*[8,9] reported that ichthyosis was not benefited by vitamin A even if a preexisting low vitamin A blood level was raised to normal by therapy. In several cases of Darier's disease, these authors reported clinical improvement on 200,000 units daily and recurrence when the vitamin was stopped. It has been reported[10] that there is deficient absorption of vitamin A in Darier's disease, but other writers[11,12] state there is no relation between vitamin A blood levels and the course of the disease.

Pityriasis rubra pilaris has been reported to improve following vitamin A therapy if a large dosage is maintained for months.[13] Familial benign chronic pemphigus, histologically a close relative of Darier's disease, has been said to improve with vitamin A.[14]

In none of these diseases has it been possible definitely to correlate the disease with low blood levels of vitamin A or carotene, or to associate clinical improvement on vitamin A administration with an increase in the blood level.[15]

In a study of the *topical* application of vitamin A, Flesch[16] found that four of six cases of ichthyosis improved markedly at the site of application of $1^1/_2$ and 3 per cent

From the Department of Dermatology, University of Maryland School of Medicine, Baltimore, Md.

*Assistant Professor of Dermatology, University of Maryland School of Medicine, Baltimore, Md.

(50,000 and 100,000 units/gram) vitamin A ointment. No benefit was noted in two cases of psoriasis similarly treated. There was no change in total vitamin A plasma levels; and, as the author notes, ". . . the effect of massive doses of vitamin A is a direct, local, and probably nonspecific drug effect upon the epidermis. It is not permissible to assume that a disease is a vitamin deficiency solely on the basis that it is benefited by large doses of a vitamin."

Since in acne vulgaris there is hyperkeratosis of the pilosebaceous follicle similar to that of vitamin A deficiency, this substance has been used by a number of investigators. Lynch and Cook[17] reported that 16 per cent of a group of cases of acne improved on 100,000 units of vitamin A daily. In another study,[18] a group of college students with acne vulgaris was treated with 100,000 units of vitamin A orally, once daily. Although 57 per cent showed improvement, four out of eight acne patients improved on placebos alone.

Corns, callouses, palmar and plantar keratoses, senile keratoses, and pachyonychia (acquired), as well as psoriasis, have all been reported to improve under large doses of vitamin A in small series of patients. The dosage of vitamin A has ranged from 100,000 units to 500,000 units daily.

Stoesser and Nelson[19] gave large doses of synthetic vitamin A (25,000 to 200,000 units daily) to ten children with infantile eczema and an allergy to fish, and presumably fish liver oil—a common source of natural vitamin A. Of the nine children who tolerated this preparation, six had marked and three had moderate improvement of the eczema with less dryness of the skin.

The principal effect of vitamin A in all these diseases has been its ability to modify the process of keratinization, and to produce a softer, more pliable horny substance. It often takes many months for vitamin A to benefit a patient and treatment must be continued thereafter; otherwise, a recurrence is likely.

We may summarize by saying that in the practice of dermatology today Vitamin A is found helpful in keratosis pilaris, keratosis follicularis, benign familial pemphigus, pityriasis rubra pilaris, in several chronic dry skin conditions and, occasionally, in acne. However, it is only helpful and not curative, and must be used along with other therapeutic measures. It probably must be continued indefinitely to maintain its beneficial effects.

Riboflavin

Sebrell and Butler[20] in 1938 reported that humans on riboflavin-deficient diets developed maceration at the corners of the mouth, with fissure formation. Greasy seborrheic accumulations were seen around the nose, eyes, and ears. These symptoms disappeared with the administration of riboflavin. A granular tongue with a painful and burning sensation may also appear in this vitamin deficiency.

However, the perlèche-like lesions described by Sebrell and Butler also may be caused by deficiencies of vitamin B_6 (pyridoxine), niacin, and iron; or the use of lipstick, dentures, gum, tooth pastes, mouth wash, cigarette holders, lozenges, and musical instruments. Only the disappearance of perlèche following the administration of vitamin B_2 and its reappearance when vitamin B_2 is withdrawn can be said to demonstrate that perlèche is due to riboflavin deficiency. A scrotal dermatitis was reported[21] in prisoners of war in the Far East as being due to riboflavin deficiency, associated with an inadequate diet. This has been confirmed recently in this country.[22]

Of 148 patients with psoriasis,[23] the lesions healed in 25 per cent, were markedly improved in 52 per cent, and improved in 16 per cent on riboflavin administration. The vitamin was given intramuscularly (5 to 10 mg. once weekly) supplemented by oral administration. As the author states, this is an incomplete study and must be considered preliminary only.

Niacin

Elvehjem *et al.*[24] first reported that niacin was specific for "black tongue" in dogs. Goldberger[25] demonstrated niacin deficiency to be the cause of pellagra. Patients with pellagra present a characteristic bilaterally symmetrical dermatitis on the dorsum of the hands, as

well as elbows, knees, neck, axillae, and perineal region. The skin is erythematous, rough, scaling, and fissured. There is sharp demarcation from the normal skin. Gastrointestinal and psychiatric disturbances may be associated with the dermatitis. Usually, niacin alone will not clear up the pellagra lesions. Associated deficiencies of thiamine and riboflavin are also present. All three vitamins are necessary, as well as a well balanced diet.

Unconfirmed work of Johnson and Binkley[26] in 1950 reported niacin was valuable in treating dermatitis herpetiformis (Dühring's disease).

Pyridoxine

Pyridoxine was isolated in 1938 and its deficiency was shown to be one of the causative factors in a skin lesion in rats known as acrodynia, although concomitant pantothenic acid and ethyl linolate deficiency is necessary to produce the disease. All three substances are necessary to clear up the acrodynia.[27] No human counterpart of acrodynia of the rat in man is known. Seborrheic dermatitis,[28] acne,[29] eczema,[30] and vulvitis[31] have all been treated with oral or parenteral pyridoxine with variable and unconfirmed results. The work of Schreiner *et al.*[32] with pyridoxine ointment points to seborrhea sicca as a metabolic defect in the skin which increases the local requirement for pyridoxine. Most dermatologists find little use for this vitamin at present, although the local use of pyridoxine ointment should be investigated further after the excellent report of Schreiner *et al.*

Pantothenic Acid

Graying of the hair of black rats with pantothenic acid deficiency was reported by several investigators and this was applied to human canities.[33,34] This research has been discredited by many investigators, and *Nutrition Reviews*[35] in 1946 scoffed at the entire work. Since then little has been heard on this subject.

Para-aminobenzoic Acid

Para-aminobenzoic acid is a chromotrichial factor in the rat and a growth-promoting factor for the chick. No definite place in human nutrition, however, can be assigned to PABA at present. It produced an immediate suppressive action in a group of patients with eczematous and atopic dermatitis.[36] It has also been used in dermatitis herpetiformis, lymphoblastoma cutis, and dermatomyositis, among other conditions.

Biotin

Biotin, a poorly understood member of vitamin B complex, when given parenterally was found to inhibit the flush following oral nicotinic acid. Although the effect of one dose (25 mg.) lasted several days, the inhibition decreased on repeated injections. The most likely explanation is that biotin may interfere with nicotinic acid absorption from the intestinal tract. Since acne rosacea may be considered an abnormal response of cutaneous capillaries to vasodilatory stimuli, biotin was administered to sixteen women. A favorable but temporary effect was noted.[37] Here again, more work is needed along these lines.

Other Fractions of Vitamin B Group

Tulipan[38] has shown that vitamin B complex is helpful in acne rosacea. Vitamin B_{12}, the newest member of the vitamin B family, is a cobalt derivative. One of its dermatologic uses is in herpes zoster where it is reported to relieve the pain of this disease rapidly.[39] Andrews *et al.*[40] claimed good results in seborrheic dermatitis with vitamin B_{12} as supplemental therapy. It is also said to have produced good to excellent results in chronic discoid lupus erythematosus, and transient but marked improvement in the lesions of one patient with subacute disseminated lupus erythematosus.[41] The dermatologic use of vitamin B_{12} at present is uncertain, and the reported value of this substance must be further studied and confirmed.

Vitamin C

There is general agreement that ascorbic acid affects the formation of normal intercellular substance. Bleeding of the gums, for example, is explained by the fact that in ascorbic acid deficiency the intercellular sub-

stance in capillary walls is faulty and the walls leak blood. Wound healing is said to be promoted by ascorbic acid; hence, it was recommended in armed forces rations[42] and in healing decubital ulcers. The anemia and the cutaneous and mucous membrane lesions of the rare Sjögren's syndrome are apparently on a vitamin C deficiency basis. This condition responds well to treatment with ascorbic acid. Some of the dyskeratoses such as keratosis pilaris and Darier's disease (keratosis follicularis), which do not respond to vitamin A, may be helped by vitamin C. On the whole, however, vitamin C is not used by most dermatologists today.

Vitamin D

The second fat-soluble vitamin discovered was vitamin D, which is necessary for the prevention and cure of rickets. In years past, vitamin D was reported to be of benefit in acne,[43] in scleroderma,[44] in psoriasis,[45] in topical treatment of x-ray burns,[46] and also in eczema.[47] The most important dermatologic use of vitamin D came with the work of Charpy[48] in France and Dowling[49] in England during World War II, in which they independently found that vitamin D_2 (irradiated ergosterol) effectively cured lupus vulgaris, a form of tuberculosis of the skin. Charpy utilized 600,000 international units twice or three times weekly in some cases. A tissue acidosis which kills the tubercle bacilli is said to result. Dowling's results were similar. Chaglassian[50] found calciferol (vitamin D_2) gave improvement in tuberculoid leprosy in doses of 600,000 units twice weekly.

The advent of streptomycin, para-amino salicylic acid, and now isonicotinic hydrazide in all types of tuberculosis has overshadowed the dramatic work with vitamin D_2.

Vitamin E

Vitamin E is found in wheat-germ oil. Chemically, it has been found to be made up of alpha, beta, gamma, and delta tocopherols. Its role in skin disorders is controversial. Vitamin E was recommended at first in chronic discoid lupus erythematosus.[51] However, in a recent report, combined oral and parenteral vitamin E therapy are stated to have given uniformly poor results. Considerable improvement was obtained by Pascher *et al.*[52] in one case of sarcoidosis and one case of radiodermatitis with ulcer. Granuloma annulare has been treated with vitamin E (300 mg. daily) with nine cures in a group of thirteen cases.[53] Although some dermatologists are convinced of the efficiency of vitamin E in these conditions, many are skeptical. It is interesting to note, however, that vitamin E was the only fat-soluble vitamin found in human sebum.[54]

Discussion

Microanalysis of fifteen samples of normal human skin revealed the presence of ascorbic acid, niacin, pantothenic acid, riboflavin, thiamine, folic acid, biotin, and cyanocobalamin (vitamin B_{12}), in that order of decreasing content.[55] It is interesting that the concentration of niacin, for example, was reported as 15 μg. per Gm. of dry skin. It is clear that the total skin contains considerable amounts of water-soluble vitamins. Nevertheless, the therapeutic value of vitamins in cutaneous disorders is limited.

Many claims of therapeutic benefit for a wide variety of dermatologic entities have been made. Gradually, as each claim has been evaluated by different workers, the original findings have in many instances failed to be confirmed or been definitely disproved. This tendency to enthusiasm persists, so that a critical attitude is necessary to determine where fact ends and fancy begins.

From my own clinical observations, the following summarizes the current practical employment of vitamins in dermatology today.

Vitamin A is helpful for the dry scaling skin alone or where it is part of the picture of ichthyosis or keratosis pilaris. In the latter entities, vitamin A must be continued indefinitely as maintenance therapy in dosage of 100,000 to 300,000 units daily. In pityriasis rubra pilaris some amelioration of symptoms occurs with vitamin A, but the disease still persists. This is also true in Darier's disease and in benign familial pemphigus. In acne

vulgaris most dermatologists are not satisfied with the effect of vitamin A alone and are forced to use other methods of therapy. In all other diseases mentioned in the literature as being benefited by vitamin A, this substance is of little or no value according to current thought; and the author agrees with this viewpoint.

Despite the many claims in the literature of the value of vitamin B complex or its various fractions in many diverse skin diseases, the fact is that nicotinic acid is helpful in pellagra, and riboflavin in some cases of perlèche. Liver extract is occasionally useful in exfoliative and seborrheic dermatitis. Most other work reported is unconfirmed and disputed. The work with pyridoxine ointment in seborrheic dermatitis is interesting, and more data are necessary.

Vitamin C has been reported of benefit in a wide variety of disorders. Actual facts disclose that vitamin C is helpful only in dyskeratotic diseases where vitamin A fails, in scorbutic purpuras, and in decubital ulcers. The anemia and the cutaneous and mucous membrane lesions of Sjögren's syndrome are benefited by vitamin C administration. Continued large amounts of the vitamin must be maintained in the chronic dermatoses. Daily dosage of 1000 mg. is not excessive.

Vitamin D has only one real dermatologic use. In the form of irradiated ergosterol or calciferol (vitamin D_2) it was found to be highly successful in the treatment of tuberculoderms—particularly lupus vulgaris, as well as in scrofuloderma and tuberculosis verrucosa cutis. Signs of hypercalcemia must be watched for in these patients.

Vitamin E has been disappointing in the treatment of discoid lupus erythematosus, in spite of the reports in the literature. In ulcers of the skin and in granuloma annulare, further investigation is in order before final judgment can be passed.

Conclusions

From this by no means complete review of the uses of vitamins in dermatology, it can be seen that in spite of enthusiastic claims, the indications for vitamin therapy are few in number. Nevertheless, there are certain diseases in which vitamin administration is clearly indicated. The author's personal experience with vitamins in dermatology is described.

References

1. Hopkins, F. G.: *Analyst* 31: 385, 1906, cited in *Vitaminology*, page 1, by Walter H. Eddy. Williams & Wilkins Co., Baltimore, Md., 1949.
2. Funk, C. A.: On the chemical nature of the substance which cures polyneuritis in birds induced by a diet of polished rice. *J. Physiol.* 43: 395, 1911.
3. Funk, C.: *Die Vitamine.* J. F. Bergman, Wiesbaden, 1914.
4. Osborne, T. B., and Mendel, L. B.: The relation of growth to the chemical constituents of the diet. *J. Biol. Chem.* 15: 311, 1913.
5. Nichols, L.: Phrynoderma—condition due to vitamin deficiency. *Indian M. Gaz.* 68: 681, 1933; *ibid.* 69: 245, 1934.
6. Frazier, C. N., and Hu, C. K.: Cutaneous lesions associated with deficiency in vitamin A in man. *Arch. Int. Med.* 48: 507, 1931; *Arch. Dermat. & Syph.* 33: 825, 1931.
7. Carleton, H., and Steven, D. M.: Keratosis follicularis; study of four cases. *Arch. Derm. & Syph.* 48: 143, 1943.
8. Peck, S. M., Glick, A. W., and Chargin, L.: Studies in cases of ichthyosis. *Arch. Derm. & Syph.* 48: 32, 1943.
9. Peck, S., Glick, A. Sabotka, H., and Chargin, L.: Vitamin studies in cases of keratosis follicularis. *Arch. Derm. & Syph.* 48: 17, 1943.
10. Ruck, D. M., Brunsting, L. A., and Osterberg, A. E.: Use of vitamin A tolerance test in certain cases of dermatologic disorders. *Proc. Staff Meet., Mayo Clinic.* 21: 209, 1946.
11. Leitner, Z. A., and Moore, T.: Vitamin A in Darier's disease. *Brit. J. Dermat.* 60: 41, 1948.
12. Moore, T.: Vitamin A. *Postgrad. M. J.* 77: 52, 1941.
13. Godding, E. W., and Porter, A. D.: Pityriasis rubra pilaris and vitamin A. *Brit. J. Dermat.* 57: 197, 1945.
14. Pinkus, H.: Familial benign chronic pemphigus. *Arch. Derm. & Syph.* 53: 119, 1946.
15. Cornbleet, T., Popper, H., and Steigman, F.: Blood vitamin A and cutaneous diseases. *Arch. Derm. & Syph.* 49: 103, 1944.
16. Flesch, P.: Inhibition of keratin formation with unsaturated compounds. *J. Invest. Dermat.* 19: 353, 1952.
17. Lynch, F. W., and Cook, C. D.: Acne vulgaris treated with vitamin A. *Arch. Derm. & Syph.* 55: 355, 1947.

18. Savitt, L. E., and Obermayer, M. E.: Treatment of acne vulgaris and senile keratoses with vitamin A: results of a clinical experiment. *J. Invest. Dermat.* 14: 283, 1950.
19. Stoesser, A. V., and Nelson, L. S.: Synthetic vitamin A in treatment of eczema in children. *Ann. Allergy* 10: 703, 1952.
20. Sebrell, W. H., and Butler, R. E.: Riboflavin deficiency in man. *Pub. Health Rep.* 53: 2282, 1938.
21. Frankland, A. W.: Deficiency scrotal dermatitis in P.O.W.'s in the Far East. *Brit. M. J.* 1: 1023, 1948.
22. Hills, O. W., Liebert, E., Steinberg, D. G., and Horwitt, M. K.: Clinical aspects of dietary depletion of riboflavin. *Arch. Int. Med.* 87: 682, 1951.
23. Maynard, M. T. L.: Psoriasis treated by riboflavin. *J. Invest. Dermat.* 18: 305, 1952.
24. Elvehjem, C. A., Madden, R., Strong, F. M., and Woolley, D. W.: Anti black tongue activity of various pyridine derivatives. *J. Biol. Chem.* 124: 715, 1938.
25. Goldberger, J., and Wheeler, J. A.: The experimental production of pellagra in human subjects by means of diet. *U. S. Hyg. Lab. Bull.* 120: 7, 1930.
26. Johnson, J., Jr., and Binkley, G. W.: Nicotinic acid therapy of dermatitis herpetiformis. *J. Invest. Dermat.* 14: 233, 1950.
27. Quackenbush, F. W., Steenbock, H., Kemmerow, G. A., and Platz, B. R.: Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis. *J. Nutrition* 24: 225, 1942.
28. Wright, C. S., Samitz, M. H., and Brown, H.: Vitamin B_6 (pyridoxine) in dermatology. *Arch. Derm. & Syph.* 47: 651, 1943.
29. Jolliffe, W., Rosenblum, L. A., and Sawhill, J.: Effects of pyridoxine on persistent adolescent acne. *J. Invest. Dermat.* 5: 143, 1942.
30. Leber, R.: Versuche der Eksembehandlung mit Adermin (Vitamin B_6). *Monatschr. f. Kinderheilk.* 88: 186, 1941.
31. Hesseltine, H. C.: Vitamin therapy in vulvar dermatosis; components of B complex. *Am. J. Obst. & Gynec.* 42: 702, 1941.
32. Schreiner, A. M., Stinger, W., Hawkins, V. R., and Vilter, R.: Seborrheic dermatitis, a local metabolic defect involving pyridoxine. *J. Lab. & Clin. Med.* 89: 948, 1951.
33. Penachavarria, A. P., Goldman, L., Herrera, C. S., and Carvajal, E. C.: Canities and alopecia in children associated with avitaminosis. *J. A. M. A.* 132: 570, 1946.
34. Brandeleone, H., Main, E., and Steele, J. M.: Effect of calcium pantothenate and para-aminobenzoic acid on the gray hair of humans. *Proc. Soc. Exper. Biol. & Med.* 53: 47, 1943.
35. *Nutrition Reviews* 4: 163, 1946.
36. Lowenthal, L. J. A.: Sodium para-aminobenzoate therapy of eczema-dermatitis. *J. Invest. Dermat.* 17: 1, 1951.
37. Kalz, F., Kral, V. A., and Forsey, R. R.: Observations on biotin. *J. Invest. Dermat.* 16: 111, 1951.
38. Tulipan, L.: Acne rosacea in vitamin B complex deficiency. *Arch. Derm. & Syph.* 56: 589, 1947.
39. Levy, I.: The use of vitamin B_{12} in the symptomatic treatment of peripheral neuropathy. A preliminary report. *Tr. Am. Neurol. A.* 75: 267, 1950.
40. Andrews, J. C., Post, C. F., and Demonkos, A. N.: Seborrheic dermatitis, supplemental treatment with vitamin B_{12}. *New York J. Med.* 50: 1921, 1950.
41. Goldblatt, S.: Treatment of lupus erythematosus with vitamin B_{12}. *J. Invest. Dermat.* 17: 303, 1951.
42. Bartlett, M. K., Jones, C. M., and Ryan, A. J.: Vitamin C and wound healing. *New England J. Med.* 226: 469, 1942.
43. Hinricksen, J., and Ivy, A. C.: Value of irradiated ergosterol in treatment of acne vulgaris. *Illinois M. J.* 74: 85, 1938.
44. Norman, G. F.: Vitamin D therapy in the treatment of scleroderma. *Geriatrics* 2: 24, 1943.
45. Ceder, E. T., and Zon, L.: Treatment of psoriasis with massive doses of crystalline vitamin D and irradiated ergosterol: a preliminary report. *Pub. Health Rep.* 52: 1580, 1937.
46. McLaughlin, R. R.: Skin vitamins. *Medical Times* 67: 303, 1939.
47. Comel, M.: Vitamins A and D topical applications. *Yearbook of Dermatology and Syphilology.*. Year Book Publishers, Chicago, 1935.
48. Charpy, M. J.: Treatment of cutaneous tuberculosis with large doses of vitamin D_2. *Ann. de dermat. et syph.* 6: 310, 1946; Treatment of lupus vulgaris with calciferol. *Clin. J.* 75: 180, 1946.
49. Dowling, G. B.: Vitamin D in treatment of cutaneous tuberculosis. *Brit. J. Dermat.* 60: 127, 1948.
50. Chaglassian, H.: Calciferol treatment of leprosy. *J. Invest. Dermat.* 10: 303, 1948.
51. Burgess, J. E., and Pritchard, J. E.: Tocopherols treatment of lupus erythematosus. *Arch. Derm. & Syph.* 57: 963, 1948.
52. Pascher, F., Sawickey, M. M., Silverberg, M. G., Braitman, M., and Kanof, N. B.: Tocopherols (vitamin E) for discoid lupus erythematosus and other dermatoses. *J. Invest. Dermat.* 17: 261, 1951.
53. Cochrane, T.: Granuloma annulare—treatment with vitamin E. *Brit. J. Dermat.* 62: 386, 1950.
54. MacKenna, R. M. B., Wheatley, V. R., and Wormall, A.: The composition of the surface skin fat ("sebum") from the human forearm. *J. Invest. Dermat.* 15: 33, 1950.

55. Lee, T. H., Lerner, A. B., and Halberg, R. J.: Water soluble vitamins in normal human skin. *J. Invest. Dermat.* 20: 19, 1953.

Resumen

Vitaminas en dermatología

A pesar de entusiastas declaraciones, las indicaciones para vitaminoterapia en dermatología son muy pocas. Sin embargo, existen ciertas enfermedades en las que la administración de vitaminas está claramente indicada.

La vitamina A es de ayuda en el tratamiento de la piel escamosa, ya sea que se presente sola o como parte del cuadro de la ictiosis o queratosis folicular. En las dos últimas entidades, la administración de vitamina A debe continuarse indefinidamente como una terapia de mantenimiento en una dosis de 100.000 a 300.000 unidades diarias. En la pitiriasis rubra pilaris se obtiene un ligero mejoramiento de los síntomas con la administración de vitamina A, pero la enfermedad todavía persiste. Esto se aplica también a la enfermedad de Darier y al pénfigo benigno familiar. En el acné vulgaris la mayoría de los dermatólogos no están satisfechos con la administración de la vitamina A en forma aislada y se ven forzados a recurrir a otros métodos terapéuticos. De acuerdo con las actuales investigaciones, la vitamina A es de ligero o ningún valor en el tratamiento de todas las demás enfermedades mencionadas en la literatura como siendo beneficiadas por esta vitamina.

A despecho de las numerosas declaraciones realizadas en la literatura con respecto del valor del complejo vitamínico B o de sus diversas fracciones en el tratamiento de muchas y diferentes enfermedades de la piel, el hecho es que el ácido nicotínico es de utilidad en la pelagra y la riboflavina en algunos casos de *perlèche*. El extracto de hígado es ocasionalmente de valor en la dermatitis exfoliativa y seborreica. La mayoría de los demás trabajos que han sido comunicados están sin confirmación o en disputa. El trabajo realizado con el unguento de piridoxina en la tratamiento de la dermatitis seborreica es interesante.

Se ha informado sobre el uso de la vitamina C en una gran variedad de desórdenes. Los hechos actuales revelan que esta vitamina es de utilidad solamente en las enfermedades disqueratósicas en las que existe una falla de vitamina A, en las púrpuras escorbúticas y en las úlceras de decúbito. La anemia y las lesiones cutáneas y mucosas del síndrome de Sjögren son beneficiadas con la administración de vitamina C. En las dermatosis crónicas se necesita administrar en forma continuada grandes dosis de vitamina C. Una dosis diaria de 1000 mg. no se considera excesiva.

La vitamina D tiene solamente una aplicación real en dermatología. En forma de ergosterol irradiado o calciferol se encontró ser de gran beneficio en el tratamiento de la dermatitis tuberculosa, especialmente en el lupus vulgaris así como en la escrofolusis cutánea y en la tuberculosis verrugosa del cutis. Con estos pacientes se debe tener paciencia y observar signos de hipercalcemia.

A despecho de los reportes publicados en la literatura, el tratamiento del lupus eritematoso discóideo con vitamina E ha sido un fracaso. Se necesitan investigaciones posteriores antes de dar una opinión final sobre el uso de la vitamina E en el tratamiento de las úlceras de la piel y lupus eritematoso.

Editorial

Research in Clinical Nutrition

The field of research in clinical nutrition is fraught with difficulties. A moment's reflection will reveal the hurdles and barriers to the apparently simple matter of obtaining data.

Unlike antibiotic research, for example (and this in no way is meant to imply that antibiotic research is *easy*), where one can pinpoint the problem, e.g., "the effect of X antibiotic on infections with Y organisms," in nutritional research we are dealing with a situation which perhaps can be described as follows:

We administer precisely known quantities (sometimes) into bodies of uncertain functional status which have evolved from years of equally uncertain stresses and nutriture; something happens inside and we can only guess what by measuring what comes out or what circulates in the blood. Sometimes we try for a higher goal and attempt to discover whether feeding something to one group of persons will produce detectable differences as compared to a similar group not so supplemented. When the variability of the human species is considered, and when the absence of unmistakable end points in our crude testing systems is appreciated, we then can understand why nutrition literature is so replete with erroneous data or misinterpreted conclusions. While it may be manifestly impossible to "control" all the countless factors impinging on the human animal, it is mandatory that every effort be expended in that direction.

This is brilliantly exemplified in the elaborate study by the U. S. Army Medical Nutrition Laboratory, the first part of which appears elsewhere in this issue.

The question that Col. Ryer and his associates attempted to answer was essentially: "Will supplementation with large amounts of vitamins B complex and C be of benefit to well-nourished, healthy persons engaged in vigorous activity under stressful conditions?" The implications are large. For example, if vitamin supplementation were to enhance physical performance, this finding would significantly alter the nutritional programs not only of the Armed Forces, but of our civilian population as well.

The meticulousness of the project can be appreciated by the description (page 99) of the "control of test subjects' food intake," Table IX (page 107), and the description of the forced marches (page 121 *et seq.*). That vitamin supplementation did not make more "iron men" out of the already rugged group of soldiers may have been expected. But what was needed was a clean-cut, definitive study, which (as the authors point out) "under the conditions of this experiment" would answer once and for all the question posed by the authors. This has been done.

Few clinical scientists have the facilities to study a nutritional problem in man with even a fraction of the cost and labor of this work. Yet all can profit from this example of careful consideration of the modifying factors in human experimentation of any type. It appears that research in clinical nutrition is impeded by great but not unsurmountable difficulties.

To the stalwart 87 enlisted men and officers who volunteered for this ten-week experiment in mid-winter in the mountains of Wyoming goes the appreciation of all of us.

—S. O. Waife, M.D.

Nutrition Briefs

RECENT ADVANCES IN EXPERIMENTAL NUTRITION

A TYPE of progressive muscular dystrophy developed in rabbits that had been fed a choline-deficient diet for some time. Hyaline degeneration was found on histologic study. Choline brought about a rapid cure within 3 days.

E. L. Hove and D. H. Copeland. *Fed. Proc.* 13: 461, 1954.

IN AN investigation of the possible therapeutic value of various agents in calciferol intoxication in mice, experimental and histologic studies indicated that neither thiol compounds, rutin, nor cortisone were able to influence experimental hypervitaminosis D_2 in these animals.

G. Polemann and G. Froitzheim. *Ztschr. f. Vitamin-, Hormon- u. Fermentforsch.* 5: 22, 1953.

RHESUS monkeys, after long exposure to moderate cold, resist intense cold better if they have been receiving 325 mg. of ascorbic acid daily. If, however, they have received only 25 mg. per day (more than the normal requirement) during the same period of exposure to moderate cold, their resistance to intense cold is not increased.

L.-P. Dugal and G. Fortier. *Gaz. méd. France* 60: 25, 1953.

HYPERVITAMINOSIS A produces serious lesions in the central nervous system of guinea pigs. Severe damage was found in the cerebral cortex, cerebellum, thalamus, and mesencephalon.

A. Brusa and F. Testa. *Intern. Ztschr. Vitaminforsch.* 25: 55, 1953.

HYPOVITAMINOSIS A in the white rat causes a reduction of alkaline phosphatase in the epiphyseal junctions of the bones. In contrast, in hypervitaminosis A, alkaline phosphatase is considerably increased only in those bones in which early ossification and fracture has occurred. In the other bones, it is reduced.

K. S. Ludwig. *Intern. Ztschr. Vitaminforsch.* 25: 98, 1953.

A HAIR growth-promoting factor for rats was found in linseed oil. Dietary fat deficiency retarded hair growth, and as little as 20 mg. linseed oil per day promoted it. This hair growth-promoting factor is stated to be linoleic acid; a similar effect is claimed for linolenic acid.

T. Rokkones. *Intern. Ztschr. Vitaminforsch.* 25: 86, 1953.

INGESTION of cholesterol and cream markedly suppressed cholesterol synthesis by the liver of monkeys. There was no change in the synthesis of cholesterol by the skin or intestine.

G. E. Cox, L. G. Nelson, W. B. Wood, and C. B. Taylor. *Fed. Proc.* 13: 31, 1954.

ESSAY CONTEST ON CLINICAL NUTRITION

The JOURNAL OF CLINICAL NUTRITION announces an essay contest open to the internes and residents of hospitals throughout the United States. This contest seeks to stimulate interest on the part of hospital medical staffs in clinical nutrition—with special emphasis on hospital aspects of nutrition. By directing the attention of the younger physician to this key subject, we believe the contest will serve the hospitals' interests, as well as provide our readers with even broader coverage of this important phase of the nutritional field.

Each paper will be carefully considered by the editorial board of the JOURNAL OF CLINICAL NUTRITION and will be judged solely on merit. Manuscripts must be received at the editorial office of the JOURNAL, 133 South 36th Street, Philadelphia 4, Pa., by July 1, 1954. Awards for the essay contest include $200 in prizes and one-year subscriptions to the first one hundred entrants. For further information, write to the Editor.

Nutritional Quotes

The Diabetic and His Diet

"Every diabetic needs to know and understand why his diet has been planned especially for him. The diet which has been given to his neighbor who is also a diabetic may not be suitable for him. The caloric value, for example, should meet the needs of the individual according to his desirable weight and activity. . . .

"The diabetic must be thought of as a person living in an environment which may affect appreciably his ability to follow instructions. He will, no doubt, have definite food preferences. His economic status will influence the type of food he can purchase. He will also be faced with some emotional problems.

"Consider what goes on in a person's mind when he first learns that he has diabetes and must remain on a diet, perhaps for the rest of his life. For a time, the bottom seems to have dropped out of everything. The whole situation involves knowing many things about the patient and his home life. . . .

"It is also important to recognize that the patient usually has a certain amount of intellectual curiosity. In the past we have acted as though we knew something which was very mysterious and as if no one but a doctor or a dietician would ever understand the calculation of the diet. However, this is no longer necessary. I think we should give the patient an opportunity to learn all about food values. In this respect a diabetic has a real opportunity to learn something his nondiabetic friends will seldom take the time to learn."

—Deaconess Maude Behrman. *Diabetes* 2: 271, 1953.

Aims and Problems of Geriatric Nutrition

"In any discussion of nutrition in relation to old age one must keep the major objective of all gerontological research in mind, namely to improve the health and happiness of the later period of life so that man may work effectively and advance the civilization in which he lives. Unfortunately many have gained the mistaken impression that researchers in old age problems are merely trying to extend the span of life and inflict upon us an ever increasing number of helpless, senile people who merely exhaust human resources and add nothing at the end of life. . . .

"Fortunately many of the problems of health and nutrition in old age are solvable by the use of test animals although one never makes quite an ultimate solution in applying results to man because he must always deal with the great human interrelationships between the mind and the body."

—Clive M. McCay. *Gastroenterologia* 80: 194, 1953.

Deadlier . . . but Not So Dead

"In the course of all these studies with white rats, one fact was evident that had long been known for the human species, namely that the female outlives the male. This was established more than a century ago by students of human statistics such as *Quetelet*. In our society today this survival of the female is of profound importance because we must be concerned with more older women than with older men. . . .

"The fact that the female of a species of experimental animals such as the white rat outlives the male gives us additional confidence that [*Roger*] *Bacon*'s thesis was correct, namely that man might study lower animals and apply his results with some degree of confidence to human beings. However this observation with animals, kept side by side in single cages in a laboratory, destroyed our confidence in the many explanations of why women outlive men. Today we have no sound explanation of this phenomenon but we do have means of studying it."

—Clive M. McCay. *Gastroenterologia* 80: 195–196, 1953.

Social Level and Diet Level of Pregnant Women

"Among the group studied in this small college town, the category of trade or business included mainly clerks or the 'white-collar worker,' and the labor group included, for the most part, only the unskilled worker. The data established a definite trend indicating that the wives in the professional and student groups had more adequate diets than those of the tradesmen or laborers. This was not a reflection of income level because the student wife with less income had a more adequate diet than the wife in the trade or labor group. . . . When the adequacy of the diet was examined in relation to the training or education of the women themselves, 42 per cent of those with the most education ate the most adequate diet, while only 22 per cent of those with no vocational or professional training past high school were in the group with the highest dietary rating. Conversely, 21 per cent of the more educated women and 43 per cent of the less educated had the lowest dietary rating. It was interesting that the average weekly amount spent per food expenditure unit was the same for the professional and laboring groups. . . ."

—G. H. Murphy and A. W. Werts. *Journal of the American Dietetic Association* 30: 34, 1954.

Progress into Complexity

"It is customary, in nutritional physiology, to estimate carbohydrate, protein, and lipid requirements by envisaging each of these three groups of nutritional principles as independent of the others. Thus we often fix the protein ration at a particular level. We then adopt a certain lipid ration, at least with

regard to the minimum judged indispensable, and finally we make up the quota of energy requirements called for with an appropriate complement of carbohydrates. And yet, the idea of optimal equilibrium and of mutual dependence between the contributions of these various nutrients has been progessively confirmed for some time now. In particular, it has been recognized that multiple factors can influence this equilibrium, exogenous as well as endogenous; among the latter. . .the neurovegetative centers and the endocrine glands. Still, precise, concrete ideas about the equilibrium between various nutrients, capable of statistical representation, are rather scarce.

"The great 'communication routes' followed by these three categories of nutrients in the course of their metabolic peregrinations, transforming themselves chemically during these transits, are beginning to be better understood. We are learning to grasp where the important crossroads are located at which they meet and where bifurcations indicate to us the mechanism of the reciprocal influences involved."
—E.-J. Bigwood. *Gaz. méd. France* 60: 9, 1953.

Nutrition in Eastern Europe

"Excessive 'cerealism,' which in some regions takes on the proportions of monoculture, explains the dominant proportion of cereals in the diet, which furnishes 70% of the total caloric requirement. This figure, which represents average consumption in four countries, necessarily masks regional aspects which are even more striking, and which approach a veritable monophagism.

"There is nothing astonishing in the fact that the major maladies of farmers and of great eaters of cereals—pellagra and beriberi—should be seasonal diseases of summer and work. In Rumania, the cases of pellagra appear during the period in which the caloric ration is highest and the alimentary imbalance most accentuated.

"Above all, however, it is the indirect biological consequences of defective diet which are most visible. In the first place, infantile mortality. . .attains. . . an extremely high figure in Eastern Europe. It must be added, finally, that the average duration of life is much shorter than in Western Europe."
—J. Claudian. *Gaz. méd. France* 60: 22, 1953.

Human Carnivores

"We may picture the true Eskimo, then, as a person who has adapted with extraordinary efficiency to subsisting in the Arctic as a typical carnivorous animal, and who is peaceful, extremely happy, and healthy (except for occasional periods of starvation). But contact with white traders, trappers, and missionaries is causing malnutriture and ill-health, as has happened extensively in the Indians."
—H. M. Sinclair. *Proceedings of the Nutrition Society* 12: 78, 1953.

Hard Work: How Hard?

"The hardest kinds of industrial work require of the human machine a caloric expenditure of some 4 to 5000 calories a day, basal metabolism included. Among woodcutters still greater expenditures are found, reaching as much as 6 and 7000 calories.

"When we realize that the supplementary caloric expenditure takes place only during the hours of work, we are even more struck by the comparison between these figures and the 2400 calories per day which are considered to be normal for light work. We are thus adding to the requirements of the basal metabolism figures of about 500 calories per hour of work. Recent estimates of respiratory metabolism carried out on Finnish woodcutters during the felling and cutting of trees have confirmed these figures."
—M. J. Karvonen. *Gaz. méd. France* 60: 35, 1953.

Pro-Cholesterol

"There unfortunately persist certain false prejudices on the subject of the relation which would seem to exist between cholesterol and diet: too often one blames on the crimes of cholesterol certain diseases such as arteriosclerosis, when in reality it is today proven that cholesterol is rather an associated or adjuvant factor. This whole series of hypotheses was never solidly supported, much less proved. Because cholesterol is the primary constituent in biliary lithiasis and is found in all the cells of the animal organism, one is too often led to hold it responsible for certain hepatic or circulatory diseases, rather than to consider it as one of the most essential nutritional elements of the organism.

"Moreover, it is considerably aided in its functions by its great solubility in all solvents. Unlike glucose in the sense that it is not a threshold substance, it is perhaps, with the latter, one of the most important substances in metabolism."
—R. Lachance. *Gaz. méd. France* 60: 38, 1953.

Need for Iron Reserves

"One occasionally hears the statement made that the need for iron is so small that it will be supplied by any normal diet and we need not worry about it. This is probably true for adult men, adolescent boys, and older women. It may be true for elementary school children for current needs, but it does not take into account the fact that they should be building up iron stores for the future. In my opinion, attention should be given to the iron content of diets for infants, preschool children, elementary school children, adolescent girls, and a certain percentage of women in the child-bearing years whose needs are relatively larger than those of other women in the same age range."
—F. A. Johnston. *Journal of the American Dietetic Association* 29: 761, 1953.

Reviews of Recent Books

Biochemistry and Physiology of Nutrition, Vol. II, edited by G. H. Bourne and G. W. Kidder, Academic Press, Inc., New York, 1953, pp. 582, $15.00.

The second of the two volumes in this international review of nutritional chemistry and physiology is equally divided among American and British authorities. Among the subjects discussed are structural changes in vitamin deficiency, coenzymes, respiratory and phosphorolytic enzymes, iron, calcium, and phosphorus metabolism, and trace elements. Dr. Goldsmith presents a concise and unusually complete review of current investigations applied to human nutrition.

Like the first volume (reviewed in these pages, Vol. 1, page 560, Nov.-Dec. 1953), this book has the chief advantage of being useful—which is, after all, the main purpose of scientific books. The multitudinous references, many surprisingly current, appear as footnotes; together with a complete author and subject index, they make the wide subject coverage conveniently available.

These volumes will prove unusually valuable to serious workers in the field. Certainly, those with an interest in physiology and biochemistry will refer to it. Clinicians, nutritionists, and dieticians will also appreciate its scope and quality. S.O.W.

Progress in the Chemistry of Fats and Other Lipids, Vol. 2, edited by R. T. Holman, W. O. Lundberg, and T. Malkin, Academic Press, Inc., New York, 1954, pp. 323, $9.80.

The chemistry and biology of lipid materials, after a slow start, has grown very rapidly in scope in recent years. This book, a collection of monographs, is an attempt to organize the accumulating data in this field.

Among the subjects covered are surface properties of fatty acid, infrared absorption spectroscopy, autooxidation of fats, and several others.

Of particular interest to our readers is the excellent review, "Nutritional Significance of the Fats" by H. J. Deuel, Jr., Los Angeles. Based on 425 references, many of recent vintage, this is a complete survey of such facets of lipid metabolism as the relationship of fat intake to protein metabolism, growth, and work capacity, effect of fat on vitamin requirements, and the relationships of fat to x-radiation thyrotoxicosis, and stress. There is an adequate discussion on digestibility, absorption, and comparative nutritive values of various fats found in average diets.

Among the conclusions is the statement that fats have beneficial effects over and above that of their essential fatty acids—a conclusion also suggested by Meng and Youmans in a recent report in this JOURNAL (J. CLIN. NUTRITION 1: 372, 1953).

This volume should prove of considerable value to nutritionists and all who are concerned with lipid chemistry. S.O.W.

Study in Human Starvation. 2. Diets and Deficiency Diseases. Map published by American Geographic Society, New York, Oct. 1953. Price $1.25 folded; $1.50 flat.

The American Geographic Society has available a most interesting map of the world distribution of deficient diets and deficiency diseases. It actually consists of 6 maps on one chart and is based on a compilation of official and unofficial data from more than 300 sources.

Although the United States and much of Europe are outstanding in being essentially "free" of serious nutritional deficiency, the widespread existence of malnutrition is striking. One need not look at darkest Africa or the remote regions of central Asia to find how much of the world subsists on an unsatisfactory food intake.

There is also included a long list of all the important bibliographic data on this aspect of geographic medicine.

This work will prove of great value to all persons interested in global nutrition and serves as a chastening reminder of the millions of ill-fed on the earth today. S.O.W.

Books received for review by the *Journal of Clinical Nutrition* are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

A Symposium on the Mechanism of Enzyme Action, edited by W. D. McElroy and B. Glass, The Johns Hopkins Press, Baltimore, 1954, pp. 816, $11.00.

Eating Together, The Diabetic Cookbook for Family Use by C. Macaulay, Farrar, Strauss & Young, Inc., New York, 1950, pp. 419, $3.95.

Food Selection and Preparation by M. D. Sweetman and J. Mackellar, John Wiley & Sons, Inc., New York, 1954, pp. 645, $6.50.

Practical Chromatography by R. C. Brimley and F. C. Barrett, Reinhold Pub. Corp., New York. 1953, pp. 128, $5.00.

Abstracts of Current Literature

ANTIBIOTICS AND NUTRITION

The influence of modern antibiotic therapy with the broad spectrum "mycins" in relation to human nutrition is proving a fertile field for clinical and experimental investigation. Several of these substances have been shown to be capable of increasing the growth rates of experimental animals. It is not known whether this effect is produced directly through some biochemical influence of the antibiotic upon tissue metabolism, or whether it is secondary to an alteration of intestinal flora. Already a favorable effect of aureomycin upon pernicious anemia in relapse has appeared. Preliminary reports have also suggested that this antibiotic favorably influences growth in premature infants, as well as in undernourished children.

Nutritional Aspects of Antibiotics. O. Mickelsen. *J. Am. Dietet. A.* 29: 221, 1953.

A comprehensive review, well documented with an extensive bibliography, outlines the role of antibiotics in promoting growth of chicks, turkey poults, swine, calves, and dogs. The method whereby antibiotics act to produce growth is still unknown. Whether they act by making available to the animal some growth factor in the diet in sufficient quantity, or whether they reduce the organisms which produce an apparently innocuous disease has not been proved.

The evaluation of antibiotics in human nutrition has only started. The most immediate result of antibiotic use is that eventually cheaper and more abundant supplies of meat may result for human consumption. Most reports on the use of antibiotics in human nutrition have been on treatment of various types of anemia. One pernicious anemia patient treated with 2 Gm. aureomycin and 2 Gm. streptomycin orally each day for 32 days showed a slow increase in the number of red cells. Three Gm. aureomycin/day to a second patient for 40 days produced an increase in hematocrit to 43 per cent. Three μg. of vitamin B_{12} and 3 Gm. aureomycin to another patient gave the best clinical and hematologic response. Intravenous injection of 600 mg. aureomycin to another patient for 20 days produced no change in blood picture. A series of papers from South Africa claim that penicillin, injected and orally, produced marked improvement in patients with megaloblastic macrocytic anemia. The most recent report from this group is that megaloblastic anemias respond to oral penicillin only when the daily dose is 200 mg. or less. Two patients showed no response to 400 mg.

Two reports claim growth-promoting effects of aureomycin fed to infants. In Italy, Perrini observed slightly greater weight gains in premature infants fed 25 mg. aureomycin/Kg. body weight than in controls. In Israel, Robinson found when 50 mg. aureomycin/Kg. body weight were given to the smaller member of each of 11 sets of premature twins, the treated twin gained faster than the untreated in all but two cases. The average weight gain of the former was 29.5 Gm./day vs. 18.0 Gm. for the controls.

Scrimshaw and associates of the Institute of Central America and Panama report on three groups of Guatemalan children supplemented with either skim milk, vitamin B_{12} (20 μg./day), or 50 mg. aureomycin daily. One group received placebos. The weight increases for the skim milk group was slightly higher than that of the placebo group; the vitamin B_{12} group showed considerably higher gains; the aureomycin

group showed the greatest—a rate twice that of the placebo group. In these groups the animal protein averaged about 5 Gm./day. A similar study with another group of children whose animal protein intake was 13 Gm./day showed no influence of vitamin B_{12} nor of the antibiotic on growth. These studies verify the action of the antibiotic on the diets made up primarily of vegetable protein. The authors comment that it is doubtful whether any dramatic effects will be seen in the United States from supplementing human diets with antibiotics, since, in most cases the animal protein content is fairly high.

On this basis, the reviewer doubts the desirability or advantage of adding vitamin B_{12} to an infant's diet which consists of a milk formula with possibly meat or egg yolk additions.—J. M. SMITH

Antibiotics in Nutrition. R. Braude, S. K. Kon, and J. W. G. Porter. *Nutr. Abstr. & Rev.* 23: 473, 1953.

In this comprehensive review, the influence of antibiotics in animal nutrition is considered in the light of evidence gained in experiments with pigs, poultry, cattle, sheep, and various laboratory animals. Differences in the growth-promoting action of the various antibiotics, the varying responses of different species of animals, and the role of the diet in determining degree of response to antibiotic supplementation are reported. Bacteriological studies are collated with growth studies.

The various theories on the mode of action of antibiotics in nutrition are reviewed. That antibiotics have a sparing effect on any of the major nutrients is unlikely. In chicks, there is evidence that growth promotion by antibiotics is due to their control of an as yet little understood "infection." There is some indication that a similar therapeutic effect may be involved in the increased growth of other animals, since pigs reared under strict hygienic conditions did not respond to antibiotic supplementation. One suggestion—the "disease level" theory—is that the degree of growth stimulation by antibiotics depends on the animals' state of health. However, an additional effect occurs when all-vegetable diets are supplemented with antibiotics: here the growth response is more marked, suggesting a vitamin-sparing action.

The ultimate value of antibiotics as feed supplements has still to be demonstrated, since it is possible that, as has sometimes occurred with antibiotic therapy in man, adaptation of the gut population may lead to the development of resistant or noxious strains. As of now, however, antibiotics are useful adjuncts in the production of pork, bacon, and poultry, and in the rearing of calves. Caution is expressed with regard to their use with breeding stock, because of the greater possibility of undesirable cumulative effects.—C.-J. HOWELL

Effect of Dietary Penicillin on the Efficiency of Protein Utilization by Chicks. H. C. Saxena, M. E. Starr, L. G. Blaylock, J. S. Carver, and J. McGinnis. *Arch. Biochem. & Biophys.* 44: 346, 1953.

In an effort to resolve some of the controversy on the efficacy of feeding antibiotics to chicks, this study undertook to investigate the problem by using nitrogen balance techniques. Day-old chicks were randomly distributed into 18 groups of 10 chicks each and fed *ad libitum* three diets which contained 15, 18, and 21 per cent protein, respectively. Diamine penicillin (1100 units/mg.) was added to the diet of 3 groups of 10 chicks each at a level of 3 p.p.m. The growth data indicate that the chicks which received the 15 per cent protein diet supplemented with penicillin were significantly smaller than those receiving the 18 or 21 per cent protein diet supplemented with penicillin. The nitrogen retention data indicate that penicillin increased the efficiency of feed utilization at all levels of protein intake. The efficiency of feed utilization was greater at 18 and 21 per cent protein levels than at the 15 per cent protein level, regardless of antibiotic supplementation. The growth response to penicillin was significant and consistent at all levels of protein studied.—M. K. HORWITT

The influence of antibiotics upon the production of vitamins by intestinal organisms may prove to be important in human nutrition. The production of certain vitamins, such as biotin, by these organisms may be decreased during antibiotic therapy. On the other hand, the utilization of fed vitamins by the intestinal flora may be diminished during such therapy. The influence of streptomycin on the B vitamins has been investigated.

Effect of Oral Administration of Streptomycin on Urinary Excretion of B Vitamins in Man. H. P. Sarett. *J. Nutrition* 47: 275, 1952.

Measurement of the urinary excretion of B vitamins or their metabolites during a period when the metabolic activity of the bacteria of the intestines is altered by streptomycin may give some idea as to whether these bacteria usually play an important role in modifying or supplementing the dietary intake of B vitamins.

The effects of oral administration of streptomycin on the urinary excretion of thiamine, riboflavin, biotin, folic acid, pyridoxine, N^1-methylnicotinamide (N^1-Me) and the 6-pyridone of N^1-Me (pyridone), and on the excretion of nicotinic acid compounds following tryptophane supplements, were studied in 6 subjects maintained for 24 to 44 days on regulated diets. After suitable control periods, streptomycin was administered orally for 8 to 22 days in doses of 1 Gm. given either 4 or 6 times daily. Within a few days, fecal cultures showed either complete or partial inhibition of growth of coliform organisms and almost complete absence of the other aerobic organisms studied.

The excretion of folic acid was not decreased during streptomycin administration and under certain

conditions appeared to be increased. Thiamine and riboflavin excretion were decreased in one subject and not markedly affected in the others. Excretion of pyridoxine compounds was determined in one subject and found to be unaffected by streptomycin. Biotin excretion was markedly decreased during streptomycin administration, and excretion of N^1-Me and pyridone. This conversion of tryptophane to nicotinic acid compounds did not appear to be affected by streptomycin administration and the concomitant change in intestinal flora. This supports the evidence that the main conversion of tryptophane to nicotinic acid takes place in the body, and not in the intestine. —B. SURE

Antibiotics and Urinary Elimination of Riboflavin. P. Montenero and G. Frongia. *Acta Vitaminologica* 6: 204, 1952.

The effect of various antibiotics on the urinary excretion of riboflavin was studied in 2 categories of subjects: one disease-free, the other with febrile diseases.

The daily administration of 2 Gm. chloramphenicol to healthy subjects produced a slight but progressive increase in urinary elimination of riboflavin, becoming significant in the second and third 5-day periods of administration. The average elimination for the whole 20-day period was not significant, compared to the initial values. In the febrile subjects, the daily administration of 2 Gm. chloramphenicol produced a progressive decrease in urinary elimination of riboflavin, which became significant in the last 10 days. The average elimination for the entire 20 days was also significant. In one of these subjects, obvious lingual signs of ariboflavinosis appeared on the 12th day of therapy.

Similarly, in healthy subjects treated with 2 Gm. aureomycin daily, a progressive increase in urinary elimination of riboflavin was observed, becoming significant only in the first and second 10 days of administration. In the subjects with febrile diseases treated with the same daily dose of aureomycin over the same period, there was a progressive decrease in riboflavin elimination, which became significant in the last 10 days. The average 20-day elimination was also significant. In one of the 4 subjects, signs of ariboflavinosis (inflammation of the labial mucosa, gastralgia, pyrrhosis) appeared on the 8th day of therapy, followed by the appearance of diarrhea with burning on defecation. In another subject, dysepithelization of the mucosa of the tongue appeared during the last days of aureomycin administration.

After streptomycin, the urinary elimination of riboflavin in healthy subjects increased slightly, but the increase was not significant for the 20-day period. In the subjects with febrile diseases, the same doses of streptomycin administered over the same period induced a decrease in urinary elimination of the vitamin with respect to the basal values, significant for the 20-day average.

In the healthy subjects, urinary elimination of riboflavin was not appreciably modified during the administration of penicillin. In subjects with febrile diseases, a decrease was noted in a few cases, but in no case were modifications in riboflavin elimination significant.

The most probable explanation for these results seems to be that the antibiotics provoke, in healthy individuals who have a large vitamin reserve, a disequilibrium in absorption and biological synthesis of riboflavin, with the result that the vitamin is insufficiently absorbed in the intestine.

On the other hand, in individuals who, due to special pathological conditions, are lacking in vitamin reserves, the introduction of the antibiotic (perhaps with destruction of the intestinal bacterial flora, which seems to be endowed with the property of producing the synthesis of riboflavin) provokes, through defective assimilation, an insufficient utilization of the vitamin and hence a decrease in its elimination in the urine—the organism lacking vitamin reserves adequate to its requirement.

In any case, the administration of antibiotics like chloramphenicol, aureomycin, and, in smaller quantities, streptomycin, induces modifications in the urinary elimination of riboflavin; the avitaminosis provoked in this way should be classed among the avitaminoses due to insufficient absorption, of which the decrease in urinary elimination represents the most evident biochemical expression.—C.-J. HOWELL

Effects of Dietary Aureomycin upon the Intestinal Microflora and the Intestinal Synthesis of Vitamin B_{12} in the Rat. K. R. Johansson, G. E. Peterson, and E. C. Dick. *J. Nutrition* 49: 135, 1952.

The feeding of aureomycin stimulated the growth of weanling male albino rats maintained on purified rations limited in methionine or vitamin B_{12} or both, but was without effect on the growth of rats fed a practical diet.

Dietary aureomycin was found to cause a rapid, statistically significant change within the intestinal microflora of the rat, particularly among the coliform, enterococcus and lactobacillus groups of bacteria. A large portion of the flora, notably coliforms, developed resistance to aureomycin. These changes occurred in fecal droppings as well as in the cecum and ileum.

Vitamin B_{12} was synthesized in the intestines of rats fed rations (with or without aureomycin) deficient in this factor. No conclusive effect of aureomycin on the concentration of vitamin B_{12} in the intestinal tract was demonstrated.—B. SURE

Effect of Dietary Antibiotics on the Growth of Chicks Receiving Suboptimum Amounts of Thiamine by Mouth or by Injection. P. E. Waibel, W. W. Cravens, and C. A. Baumann. *J. Nutrition* 50: 441, 1953.

Penicillin, aureomycin, or a mixture of antibiotics markedly increased the growth of chicks fed diets

containing limited amounts of thiamine. The effect was similar whether the dietary carbohydrate was sucrose or dextrin. When the level of thiamine in the diet was adequate, the antibiotics increased growth only slightly. Dietary antibiotics failed to increase the growth of chicks given suboptimum amounts of thiamine by subcutaneous injection. Variations in weight within groups were much less in the injected birds than in those receiving suboptimum amounts of thiamine in the diet.

These data support the concept that antibiotics stimulate growth by suppressing nutrient-utilizing micro-organisms in the intestinal tract.—B. SURE

Considerable information on the nutritional and metabolic aspects of the antibiotics may be gleaned from the study of their influence upon bacteria. Certain amino acids have been found to influence the sensitivity of bacteria to antibiotics.

Critical Role of Amino Acids on the Sensitivity and Development of Resistance to Polymyxin B. G. J. Haas and M. G. Sevag. *Arch. Biochem. & Biophys.* 43: 11, 1953.

This report emphasizes the importance of nutritional and metabolic environment for the susceptibility of bacteria to antibiotics.

The differential influence of simple and complex media on the susceptibility of *Aerobacter aerogenes* to different concentrations of polmyxin B was studied by comparing growth in salt-glucose, casein hydrolysate, and broth media. The bacteria were most susceptible in the rich broth medium and least susceptible in the salt-glucose medium. *A. aerogenes* grown in salt-glucose before contact with the drug can grow in approximately one hundred times the amount of polymyxin as the organism subjected to the drug in broth. *Pseudomonas aeruginosa* and *Escherichia coli* were also more susceptible in richer media. In contrast, *Salmonella paratyphi B* is more resistant in richer media than in salt-glucose media.

Attempts to explain the differences in activity by adding varying combinations of serine, leucine, and methionine to salt-glucose media indicated that amino acids can prevent the emergence of resistant cells. Any two of these three amino acids when added to salt-glucose media were equal in potency to the complex broth or complete amino acid media in preventing the manifestation of the resistance phenomenon in salt-glucose media shown by *Aerobacter aerogenes*.—M. K. HORWITT

RIBOFLAVIN

Riboflavin is important as an intracellular oxidative catalyst in the form of flavoprotein. It is present in the tissues as a prosthetic group of cytochrome C reductase, d-amino acid oxidase, and xanthine oxidase. Measurement of the urinary excretion of riboflavin as a method for determining nitrogen losses from the body has been studied. In the following report, the interrelationship between riboflavin and the conversion of tryptophane to nicotinic acid is presented.

The Relation between Riboflavin and Tryptophan Metabolism, Studies in the Rat. F. Charconnet-Harding, C. E. Dalgleish, and A. Neuberger. *Biochem. J.* 53: 513, 1953.

The function of riboflavin in tryptophane metabolism is discussed in relation to the urinary metabolites excreted after feeding tryptophane and anthranilic acid to normal and riboflavin-deficient rats. The rats excreted anthranilic acid partly unchanged, partly as the glucoronide, and partly as amino-hippuric acid. In addition to the anthranilic acid metabolites, tryptophane was excreted in the urine of the riboflavin-deficient rats as acetylkinurenine, kynurenic acid, and xanthurenic acid. These deficient rats excreted much larger amounts of xanthurenic acid than normal rats. The administration of riboflavin caused an immediate return to normal excretions of xanthurenic acid. The urine of rats deficient in both riboflavin and pyridoxine contained no appreciable amounts of the anthranilic acid metabolites. It has previously been shown that riboflavin and pyridoxine functioned at some stage in the conversion of kynurenine to 3-hydroxyanthranilic acid. Pyridoxine deficiency is known to produce its effect by the inhibition of kynureninase, for which pyridoxal phosphate is the co-enzyme.

The student of nutrition who is interested in the conversion of tryptophane to nicotinic acid will find this paper useful for the excellent tabulation of the known material on this subject. Of special interest is the detailed discussion of the paper chromatographic techniques developed to isolate and identify the urinary metabolites of tryptophane. (A report by M. Mason on the "Metabolism of Tryptophan in Riboflavin-Deficient Rats," *J. Biol. Chem.* 201: 513, 1953, gives similar information.)—M. K. HORWITT

The ability of atabrine to inhibit the enzymes for which riboflavin is the prosthetic group has led to the demonstration that this drug exerts a sparing action on riboflavin in the organism. Under the conditions of the following experiment, increased riboflavin excretion is not associated with increased urinary excretion of nitrogen.

The Riboflavin-Sparing Action of Atabrine. K. Guggenheim and R. Shamir-Zernik. *J. Biol. Chem.* 202: 331, 1953.

Experiments carried out on rat tissues have shown that the antimalarial drug atabrine inhibits *d*-amino acid oxidase and diaphorase and that it prevents the combination of the apo-enzyme of cytochrome reductase with riboflavin phosphate. These inhibitions are competitively antagonized by flavin-adenine nucleotide or by riboflavin phosphate. This paper re-

ports the results of a study on the effect of chronic atabrine administration on the metabolism of riboflavin in the intact rat. Atabrine (fed at a level of 400 mg. per kilo of diet) improved the growth of rats which received less than 5 μg. of riboflavin per day. At higher doses of riboflavin in the diet there was no apparent effect of the added atabrine. The amount of riboflavin in liver and muscle, which represents about two-thirds of the total riboflavin of the organism, was not reduced even after prolonged atabrine treatment. The atabrine-treated rats after 2 and 4 weeks on the diet, excreted 2 to 3 times as much riboflavin in the urine as the controls receiving the same amount of riboflavin but no atabrine. The feces of the atabrine-treated and untreated rats contained equal amounts of riboflavin, indicating that the synthesis of intestinal riboflavin was not responsible for the increase in urinary excretion. The authors interpret these observations as indicating that atabrine exerts a sparing action on riboflavin in the organism. This conclusion is supported by the observation that the increased riboflavin excretion is not associated with an increased urinary excretion of nitrogen.—M. K. Horwitt

Further studies on the clinical and laboratory aspects of riboflavin nutrition are presented in the following papers.

Riboflavin in Human Serum. K. Suvarnakich, G. V. Mann, and F. J. Stare. *J. Nutrition* 47: 105, 1952.

The riboflavin content of normal human serum was investigated. In 141 normal well-nourished persons, free serum riboflavin and flavin-adenine-dinucleotide (FAD) were 0.84 and 2.32 μg. per cent, with standard deviations of 0.71 and 0.42 μg. per cent, respectively. There were no differences between sexes and age groups. The effect of daily riboflavin supplements has been studied, with no conclusive results. During 7 days of riboflavin supplements, serum riboflavin levels appeared higher than the initial level, but the difference was not statistically significant. However, the period of experimentation may not have been long enough to answer this question adequately.

Free serum riboflavin in diabetics, and serum FAD in persons with cardiac affections and hypertension, were observed to be higher than normal but no check was made as to how long these persons had been taking vitamin supplements regularly, which may have influenced the high concentration of riboflavin in the serum. Another factor which was not determined was the length of time between the last meal and the bleeding time. The authors admit that it is possible that the high serum riboflavin levels observed in this series of analyses were due to riboflavin intake shortly preceding bleeding.

It was found that nitrogen balance and riboflavin intake had no appreciable influence on the serum riboflavin levels, provided that bleedings were done several hours after the intake of riboflavin.—B. Sure

Riboflavin Metabolism of Women on Controlled Diets. M. L. Wu, E. Warren, and C. A. Storvick. *J. Nutrition* 51: 231, 1953.

This is a report of a study to determine the variation in daily fasting serum riboflavin, as well as in urinary riboflavin and creatinine, of 7 adult women on a controlled diet. Daily fasting values averaged 3.21 μg. per cent for 208 determinations of serum total riboflavin and 1.41 μg. per cent for 207 determinations of serum-free riboflavin. Urinary excretions of riboflavin varied from 315 to 467 μg. per 24 hours and ranged from 27 to 40 per cent of the riboflavin intake. The effects of an oral test dose of riboflavin were studied. The concentration of riboflavin in the serum reached a peak in one-half to one hour after the test dose and approached the fasting level in 5 hours. The peak in urinary excretion of riboflavin occurred one hour after the test dose and by the fifth hour the excretion of this vitamin was about the same as the one-hour fasting excretion. Based on the excretion of riboflavin in terms of micrograms of urinary riboflavin per gram of urinary creatinine, the results of this study indicate that for 2 of 7 subjects, 1.2 mg. per day of riboflavin may have been inadequate under the experimental conditions which obtained.—B. Sure

The Transport of Riboflavin by Human Placenta. J. E. Lust, D. D. Hagerman, and C. A. Villee. *J. Clin. Investigation* 33: 38, 1954.

Using a photofluorometric method for the determination of riboflavin, it was found that there was significantly more free riboflavin (and significantly less flavin adenine dinucleotide) in fetal blood than in maternal blood. The total amount of riboflavin, both free and in the nucleotide form, is greater in fetal than maternal blood. Evidence is presented suggesting that the placenta transfers riboflavin from the maternal to the fetal circulation by converting the flavin adenine dinucleotide in the maternal blood to free riboflavin and secreting the latter across the placenta into the fetus.—S. O. Waife

An interesting contribution on the effect of heart failure upon riboflavin metabolism indicates that the urinary losses of this vitamin during decompensation are reduced and that the administration of digitalis produces a prompt rise in riboflavin elimination. It is suggested that heart failure may increase the demand for flavoprotein in cellular metabolism.

Urinary Elimination of Total Riboflavin during Cardiac Decompensation: Influence of Digitalis Preparations on Urinary Riboflavin in Decompensation. P. Montenero and R. Boldrini. *Acta Vitaminol.* 6: 211, 1952.

Cardiac decompensation is accompanied by altered oxygen metabolism. Since normal oxygen metabolism depends on the proper functioning of the oxide-reducing enzyme systems, including enzymes whose prosthetic group contains riboflavin, the authors studied

the influence of cardiocirculatory decompensation on urinary elimination of this vitamin.

Seven subjects were studied: 4 with rheumatic heart disease, 2 with arteriosclerosis, 1 with cor pulmonale. Urinary riboflavin determinations were done on admission and daily for 15 consecutive days. During this time the patients were on a qualitatively and quantitatively constant diet which supplied the normal vitamin requirement. Urinary elimination of vitamin B_2 was compared to the normal of 0.61 to 1.02 mg. per day (established by the Medical Clinic of Rome as normal for healthy individuals on a standard regimen containing approximately 1.5 mg. of riboflavin).

It was found that the urinary elimination of riboflavin was always lower during cardiac decompensation than the average for the normal individual; that improvement in the state of decompensation was accompanied by a progressive and constant increase in the amount of riboflavin eliminated; that treatment with digitalis preparations led to an abrupt increase in riboflavin elimination; and that blood lactic and pyruvic acid levels were inversely related to the amount of riboflavin eliminated in the urine.

The uniformity of the results obtained in all the subjects studied clearly indicates a disturbance of riboflavin metabolism in cardiocirculatory decompensation—a disturbance which may be only one aspect of an altered metabolism of various B-group vitamins. In the same pathologic conditions, for example, there is a deficient urinary elimination of thiamine, even after loading, and the authors speculate that decompensation may increase the demand for flavoproteins, or that the biochemical imbalance typical of this condition may cause an alteration in the mechanism of respiratory flavoprotein action.

In the same condition, digitalis, shown here to increase the urinary elimination of riboflavin, decreases that of thiamine—suggesting that under the influence of digitalis, the phosphorus radicals are used preferably for the phosphorylation of thiamine. The previous finding that phosphorylated thiamine checks the elimination of riboflavin during cardiac decompensation would seem to support this hypothesis.—C.-J. HOWELL.

No effect of riboflavin administration upon blood lactic and pyruvic acid could be demonstrated in the following investigation. However, there was no evidence that the subjects studied were deficient in this vitamin. The administration of the vitamin above the individual's requirement should not be expected to alter carbohydrate metabolism in any way. It should only lead to increased urinary excretion of the vitamin.

Variations in Blood Lactic and Pyruvic Acids under the Influence of Vitamin B_2. V. Boccardelli and M. d'Adamo. *Acta Vitaminol.* 6: 215, 1952.

While the role of thiamine in glucose metabolism has been the subject of numerous studies, that of riboflavin is not so well understood. The authors therefore undertook to investigate the influence of riboflavin on pyruvic and lactic acid levels in pathological conditions where an altered glucose metabolism could be anticipated.

Fifteen patients with diabetes, heart disease, and hepato-colecystitis were selected for the experiment, and blood levels of lactic and pyruvic acids were determined before and 2 hours after intravenous administration of 1 mg. of riboflavin. Some subjects underwent the treatment for 6 days. The authors were especially interested in the interrelationship between lactic and pyruvic acids, since in subjects deficient in thiamine, the difference between the actual and calculated values of pyruvic acid (PA excess) might furnish an early clue to the degree of thiamine deficiency.

The initial values of both lactic and pyruvic acids were already rather high in these patients, suggesting a disturbance of glucose metabolism. Although the administration of riboflavin tended to lower the values of both acids, a subsequent tendency toward re-elevation was observed, even when the treatment was continued. However, the initial levels were not reached except in two cases: in one, a higher than initial level of lactic acid was attained on the 6th day; in the other, a similar phenomenon was observed in the pyruvic acid level.

In studying the lactic/pyruvic acid ratio, an excess of pyruvic acid over the theoretically calculated value was found in the majority of cases, and this ratio remained practically unchanged by administration of riboflavin. Though this vitamin is thought to be related to that phase of glucose metabolism in which lactic and pyruvic acids are intimately linked, no obvious influence of riboflavin on the disturbed glucose metabolism, as revealed by the blood lactic and pyruvic acid levels, could be observed.

Despite these negative results, the authors do not exclude the possibility that riboflavin may be helpful in regulating glucose metabolism in various pathological conditions, expecially when it is given together with larger doses of thiamine or of cocarboxylase.—C.-J. HOWELL

THIAMINE

Methods for determining the state of thiamine nutrition include the measurement of the urinary excretion of the vitamin following a loading dose, the measurement of urinary pyramin, a thiamine metabolite, and more complicated procedures such as measurement of blood or tissue free and total thiamine. Based on excretion studies, an intake of 0.5 mg. of thiamine per 1000 calories is considered adequate in human nutrition. The following study represents a careful evaluation of thiamine nutrition in normal

subjects. In this study, the ratio of thiamine to creatinine was fairly constant, so that creatinine excretion may be used as a rough estimate of the status of thiamine nutrition in these subjects.

Thiamine Metabolism of Women on Controlled Diets. I. Daily Urinary Thiamine Excretion and its Relation to Creatinine Excretion. H. A. Louhi, H. Yü, B. E. Hawthorne, and C. A. Storvick. *J. Nutrition* 48: 297, 1952.

The determination of urinary thiamine excretion has been used in many laboratories in assessing the thiamine nutrition of man, since the picture it gives is fairly reliable. There were 4 subjects for the 1949 study, 3 subjects for the 1950 study, and 4 subjects for the 1951 study. The subjects were women graduate students and one staff member, and ranged in age from 24 to 44 years. Three of them were Chinese and one Korean. Urinary thiamine was determined by a modification of the thiochrome method of Hennessy and Cerecedo and creatinine by the method of Folin.

When 8 normal women were maintained on an intake of 500 μg. of thiamine per 1000 cal., the average daily urinary excretions of thiamine ranged from 100 to 276 μg. When the thiamine intake was reduced to 300 μg. of thiamine per 1000 cal. the average daily urinary excretions of thiamine ranged from 34 to 109 μg. The thiamine excretion expressed as per cent of thiamine intake ranged from 10.0 to 27.6 for the higher intake period and from 5.7 to 18.2 for the lower intake period. Urinary thiamine excretions expressed in terms of micrograms of thiamine per gram of urinary creatinine ranged from 80 to 276 for the first period, and from 26 to 107 for the second period of intake.

Using an excretion of 100 μg. of thiamine per 24 hours, an excretion of 13 per cent of the daily thiamine intake and an excretion of 150 μg. of thiamine per gram of creatinine as indications of good nutrition with respect to thiamine, an intake of 500 μg. of thiamine per 1000 cal. was judged to be adequate for 6 of the 8 subjects and borderline for the other two. An intake of 300 μg. of thiamine per 1000 cal. was inadequate for 6 of the subjects and borderline for the other two.

The response of 4 subjects to a 5-mg. oral test dose of thiamine hydrochloride given on the last day of the period of lowered thiamine intake indicated that the tissues of all the subjects were low in thiamine.

The determination of thiamine in micrograms per gram of creatinine in individual voidings seemed to indicate that the ratio of thiamine to creatinine is fairly constant and, except in 2 of 34 instances, could have been used as well as the 24-hour ratio for a rough estimation of the status of thiamine nutrition of the subjects.—B. Sure

Thiamine Metabolism of Women on Controlled Diets. IV. Comparison of the Daily Levels of Thiamine in the Blood and Urine. E. C. Johnson, R. B. Dube, H. A. Louhi, H.-H. Yü, V. C. Wilmot, and C. A. Storvick. *J. Am. Dietet. A.* 29: 41, 1953.

The authors review the literature concerning the relative value of blood thiamine, urinary thiamine, and fasting blood pyruvic acid as measures of the thiamine nutrition of individuals. Data (reported in previous publications from this laboratory) on urinary and blood thiamine of 8 subjects consuming 500 or 300 μg. thiamine/1000 cal. are compared to see whether a correlation exists between daily values for total thiamine in whole blood and the daily urinary thiamine excretion of normal human subjects on controlled diets. This comparison revealed no significant relationship. The physiologic relationship of the two measures is discussed and the authors conclude that for clinical purposes, the most informative and practical measure of thiamine nutrition is the urinary excretion of thiamine.—J. M. Smith

Very few data exist in the literature concerning the fluctuations in blood thiamine under controlled conditions. The determination of blood thiamine following an oral dose of this vitamin may give important information concerning its absorption and utilization in various disease states.

Thiamine Metabolism of Women on Controlled Diets. II. Daily Blood Thiamine Values. R. B. Dubé, E. C. Johnson, H. Yü, and C. A. Storvick. *J. Nutrition* 48: 307, 1952.

No report has been found in the literature in which daily fasting blood thiamine values have been determined for subjects whose thiamine and caloric intakes have been known and controlled for extended periods. It was felt desirable to know how greatly the levels of thiamine in the blood fluctuate under controlled conditions. This paper is a report of the values obtained for thiamine in whole blood and of calculated values of thiamine in packed blood cells. In addition, the experiment was planned to determine whether any change in blood thiamine concentration would occur when the thiamine intake was reduced from 500 μg. to 300 μg. per 1000 cal. Some determinations of blood thiamine were also made on 2 subjects on controlled diets but ingesting therapeutic amounts of thiamine, on 2 ambulatory patients receiving thiamine therapy, and on 5 normal people having self-selected diets.

Eight women served as subjects in studies designed to follow the daily fluctuations of thiamine in whole blood when the thiamine intake was controlled at 500 and 300 μg. of thiamine per 1000 cal. for periods of two weeks or more. The diet was constant within each study, the only variable being the level of thiamine from one period to the other. With an intake of about 500 μg. of thiamine per 1000 cal. the average fasting blood thiamine ranged from 3.3 to 5.4 μg. per 100 ml. of whole blood or 7.0 to 11.2 μg. per 100

ml. of packed cells. When the thiamine intake was lowered to 300 μg. of thiamine per 1000 cal. the average fasting blood thiamine ranged from 2.8 to 4.6 μg. per 100 ml. of whole blood or 6.3 to 10.0 μg. per 100 ml. of packed cells. The effect of a 5-mg. oral test dose of thiamine hydrochloride on the level of thiamine in the blood is presented. A few values for blood thiamine obtained for people other than experimental subjects are included.—B. SURE

Patients with long-standing congestive heart failure have been shown to be deficient in thiamine, based on tests performed by the loading dose method. One factor involved in the hypothiaminosis detected in these patients may be the enhanced rate of thiamine excretion which has been observed during a period of mercurial-induced diuresis.

Effect of Mercurials on Thiamine Excretion in Patients with Congestive Heart Failure. M. G. Wohl, C. R. Shuman, and R. Turner. *Proc. Soc. Exper. Biol. & Med.* 83: 323, 1953.

Thiamine excretion and urinary output were correlated in patients with congestive heart failure receiving mercurial diuretics. None of these patients had clinical evidence of thiamine deficiency before or during the administration of mercurials. All patients studied had a significant increase in thiamine excretion during the period of mercurial administration. This increase did not appear to be directly correlated with the increased urinary volume. The authors postulate that it may be related to depressed renal tubular reabsorption of thiamine.—L. W. KINSELL

Thiamine employed in the treatment of acute beriberi was found to result in hypertension which was thought to aggravate the cardiac difficulties present in these patients.

Hyperpiesis in Cardiovascular Beri-Beri. J. H. Walters. *Quart. J. Med.* (New Series) 22: 195, 1953.

Eight patients form the basis of this report. All were pearl divers in the Persian Gulf, who ate a diet adequate in total calories but unbalanced in respect to thiamine/nonfat-calorie ratio. All rather suddenly developed anasarca, hypertension, cardiomegaly, myocardial damage, and no or minimal signs of peripheral neuritis. Evidence is presented suggesting that this was due to beriberi with unusual cardiovascular manifestations. Treatment with thiamine led to an increase in blood pressure. Heart failure due to beriberi is of the high-output type and has as its mechanism general arteriolar dilatation and myocardial weakness. Thiamine led to rapid arteriolar constriction and may have aggravated the cardiac difficulties. In at least one patient who survived, irreparable myocardial damage persisted despite recovery from the acute stage of cardiovascular failure.—S. O. WAIFE

ITEMS OF GENERAL INTEREST

Some Current Aspects of Nutrition. J. B. Youmans. *J. Am. Dietet. A.* 28: 1029, 1952.

This article reviews some of the problems in clinical nutrition, as well as aspects of nutrition concerned with public health and the nutrition of populations. With a steady loss of emphasis by physicians on nutritional deficiency diseases, there has been increased attention to nutritional implications of heart failure, edema, hypertension, renal failure, and ascites. Nutritional problems in the use of antibiotics and parenteral feeding, in arteriosclerosis, and in aging have commanded attention.

In the United States there is less concern over nutritional deficiencies than over overnutrition, with ever-increasing interest in obesity. Discovery of an unusual type of fat molecule in the serum of arteriosclerotic patients has led to indictment of a number of fat- (or cholesterol-) rich foods. Nutritionally important foods such as eggs, milk, cream, and butter may be prohibited. This accomplishes caloric restriction at the expense of other important nutrients.

The popularity of low sodium diets in the treatment of hypertension, congestive heart failure, and other conditions has had its drawbacks. Recently, attention has been called to the occurrence of sodium deficiency in individuals on low sodium diets. This may be exaggerated by excessive fluid intake, parenteral glucose, or mercurial diuretics. Sodium deficiency may result in depressed urine output, electrolytic imbalance, acidosis, renal shutdown, and death. Use of exchange resins may result in deficiencies of sodium and other minerals (potassium and calcium). Magnesium, iron, and some vitamins may be removed by cation-exchange resins.

Potassium has been receiving attention lately. Deficiencies may occur in both acute and chronic forms. An acute deficiency usually occurs with increased losses in urine and stools when intake is restricted, conditions found in trauma after operations, with drainage of body fluids, gastrointestinal suction, vomiting, and acute diarrhea. Use of cortisone and ACTH may produce a similar deficiency. The lassitude and weakness associated with chronic illness and with convalescence are considered due to potassium deficiency. Potassium excess results in symptoms indistinguishable from deficiency.

Parenteral feeding is a large area of clinical interest in nutrition. Adequate nutrition requires sufficient calories and protein over relatively long periods. Currently, amino acids are being used, but one of the principal errors is failure to provide sufficient calories to prevent use of amino acids for energy. Glucose in too concentrated solutions is irritating and causes thrombosis. More dilute solutions involve intake of large amounts of water which may induce congestive failure. Fat administered in the form of emulsions would alleviate some of the problems, but to date

satisfactory emulsions have not been endorsed for clinical use.

The effect of administration of antibiotics on nutrition is a relatively new problem. The role of intestinal bacteria in production of vitamins for use of man has not been determined, but the possibility exists that antibiotics, by suppressing intestinal bacterial growth and action, may induce deficiencies. The overgrowth of certain yeasts and molds may produce deficiency by competing for orally administered thiamine.

In considering the relation of nutrition to senility and the aging process, the author discusses some possible effects of more rapid growth in children on onset of senescence, pointing out that greater calorie intake in the 19th century resulted in more rapid growth of children, as well as in greatly increased and unsatisfied requirements for vitamin D and widespread rickets. Whether simple abundance of calories, permitting greater and more rapid growth, may cause changes to be reflected in earlier senescence is unknown. Possible disturbances in intestinal absorption, changes in intestinal flora, and modification in the metabolism of certain essential amino acids are additional avenues for further research.

World War II, with its effect on the food intake of many population groups, afforded opportunity to study speed of recovery from effects of reduced food. Trémolières and Boulenger found maximum growth delay in 13-year-old French girls, but by the time these girls were 16, the deficit had disappeared. The average heights of all age groups of these French children were greater in 1948, some two years after the war, than before the war. These experiences have served to emphasize the body's remarkable power of adaptation to undernutrition. Savings may be made by temporarily delayed growth in children, loss in weight in adults, lowered metabolic rate, or decreased physical exertion.

In times of food shortage, distribution must be controlled, and this in turn requires determination of nutritional status. Measurement of body weight and height is considered the best single criterion of nutritional status of populations at mild to moderate levels of food restriction. Standards of height and weight are needed, and measures of skin and subcutaneous tissue thickness may prove helpful. Unfamiliar food or a food of artificial rather than natural origin may lead to voluntary food restriction by populations.—J. M. Smith

Phosphorus Compounds in Nutrition and in Industry. E. B. Hart. *J. Am. Dietet. A.* 28: 1036, 1952.

This article, which states there are a host of phosphorus compounds (inorganic) which find a useful place in many food mixes, food processing operations, and rations of domestic animals, was written in answer to a *Consumer's Research Bulletin* article titled: "Phosphates and Phosphoric Acid: Undesirable Food Ingredients." The latter article presented quotations to point out the importance and harm of (a) an unfavorable calcium-phosphorus ratio, (b) the possibility of anemia from the formation of unavailable iron phosphate, (c) an excessive intake of phosphorus, causing renal lesions, and (d) high phosphorus ingestion causing a decreased amount of phosphorus in muscle.

In reply, Hart points out that the unfavorable effect of calcium-phosphorus ratio in feeds or foods is not often experienced in practice, though it can be demonstrated in laboratories with synthetic rations. Acid beverages may erode teeth if high in phosphates and consumed in large amounts, but from a practical standpoint it seems that the buffering effect of human saliva, together with the mucous film on the teeth, can act in many, if not most cases as a protective agent.

Phosphates (bone meal or defluorinated rock phosphate) have saved the cattle industry in areas where phosphorus deficiency occurs and have been preferred for the baby chick during the first three weeks of life because phosphorus from plant material is less available.

Disodium phosphate added to milk during evaporation prevents coagulation and process cheese is made possible through use of phosphates and salts of inorganic acids. Baking powders contain carbonate and a compound to liberate the carbon dioxide gas. Calcium acid phosphate is used for this purpose and the residue left is sodium calcium phosphate—a harmless compound.

Experimental experience gained 20 years ago shows that ferric pyrophosphate and ferric hypophosphite yield iron for hemoglobin as readily as ferric chloride or ferrous sulfate. Today, sodium-iron pyrophosphate is used for flour enrichment and ferric pyrophosphate for rice enrichment without resultant anemia.

Phosphates play a fundamental role in muscle metabolism. Muscle contraction is related to carbohydrate metabolism which does not proceed in the absence of phosphates. Many water-soluble vitamins must be phosphorylated to be active and fats are linked to phosphate before absorption through the intestinal wall. The author believes that the amounts of phosphate added to foods during processing is so small as to have no practical effect on calcium/phosphorus ratio.—J. M. Smith

The Effect of Diet on Citrulline Synthesis in Vitro. S. B. Koritz and P. P. Cohen. *J. Biol. Chem.* 200: 551, 1953.

This paper is a contribution to the growing literature on the changes which occur in the enzyme systems of the liver when the diet is deficient in protein. Using rats, the authors determined that a 12 per cent casein diet caused a greater decrease in citrulline synthesis in the presence of glutamate than in the presence of carbamylglutamate in the liver of rats. Urea synthesis from citrulline was not affected. It

has been reported that arginase is sensitive to the level of protein in the diet, but possibly due to the high concentration of arginase in the rat liver, it is not likely to be decreased by a 12 per cent casein diet to a concentration which becomes rate-limiting for urea synthesis.

When the 12 per cent casein diets were supplemented with small amounts of carbamylglutamate, a stimulation of citrulline synthesis resulted. This stimulation was even more pronounced in the presence of glutamate. The observed effect of dietary carbamlyglutamate suggests that this substance is of physiological significance.—M. K. Horwitt

Electrophoretic and Cholesterol Studies in Peptic Ulcer. H. A. Rafsky, C. I. Krieger, A. P. Boleman, and J. C. Rafsky. *Gastroenterology* 23: 363, 1953.

Studies made on 70 ulcer patients revealed a lowering of absolute serum albumin levels in 43 per cent and a lowering of the albumin/globulin ratio in 52 per cent of the cases. These changes were related to aging, complications of the disease, and other accompanying diseases. Seventy-four per cent of patients had a free cholesterol of 30 per cent or higher. No mention was made of dietary factors nor were the data analyzed statistically.—S. H. Lorber